The effect of elevated CO_2 levels on the growth of two *Acacia* species

by

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Declaration

I certify that the research work reported on in this thesis is the result of my own original investigation, except where the works of others are acknowledged.

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We the undersigned certify that the above is correct

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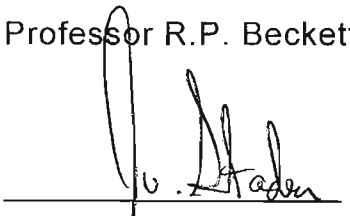 Co-supervisor

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Abstract

Climate change, induced by increases in the concentration of greenhouse gases in the atmosphere, can affect the growth and community structure of ecosystems in two ways. Firstly directly through changes in atmospheric concentration of CO_2, and secondly indirectly through changes in temperature and rainfall. The aim of the present investigation was to test the effect of elevated CO_2 and altitude-related temperature differences on the growth of two species of *Acacia* that form important components of the vegetation of KwaZulu-Natal.

Plants of *Acacia sieberana* and *Acacia nilotica* were grown in chambers at elevated (700 $\mu l l^{-1}$) and ambient (350 $\mu l l^{-1}$) CO_2 with and without rhizobial inoculation. Both treatments (elevated CO_2 and the presence of rhizobial inoculation) stimulated growth and branching. *A. nilotica* was the most responsive to both elevated CO_2 level and inoculation. Inoculated plants showed greater increases in mass and height than uninoculated plants. While elevated CO_2 had a significant effect on plant mass, height and leaf area accumulation, other factors, such as species type and rhizobial inoculation had a somewhat greater influence on the short term mass accumulation under elevated CO_2. Significant differences existed between the average percentage leaf nitrogen for the two species ($P < 0.001$), and for inoculated and uninoculated plants ($P < 0.005$).

There were no significant differences in photosynthetic rates (A) at any internal CO_2 concentration (C_i) between plants grown in elevated CO_2 compared to those grown under ambient conditions. When photosynthesis was plotted against C_i (A/C_i), the initial slopes of the graphs for both *A. sieberana* and *A. nilotica* were shallower for plants grown in elevated CO_2, compared to plants grown in ambient conditions, indicating a decreased Rubisco concentration at low C_i, and greater nitrogen use efficiency. At higher C_i, *A. sieberana* continued to have lower A in plants grown at elevated CO_2 levels suggesting an inability to regenerate RuBP or the possible accumulation of soluble carbohydrates. *A. nilotica* grown in elevated CO_2 had a slightly increased P_i regeneration capacity at higher CO_2 concentrations. While the A/C_i results demonstrate that CO_2 has a minor effect on photosynthesis, growth responses indicated otherwise. This is a result often reported and indicates the

importance of measuring as many parameters as is possible to determine actual plant responses to elevated CO_2 levels.

In the field experiment, the effect of temperature was studied by transplanting twenty plants of each species at three different elevations in the Drakensberg at Cathedral Peak. Plant height, mass, condition and finally survivorship were measured. All of these attributes decreased as elevation increased. Plants growing at the highest elevation all died back prior to winter while those growing at lower elevations grew throughout the experimental period. Results suggest that elevation and hence temperature are important factors controlling *Acacia* distribution. If the greenhouse gas induced increases in temperature occur as predicted, and the estimated latitudinal migration rates of 30-100km per decade are required for species to remain within their current climatic envelopes, it is expected that the structure and appearance of vegetation in the Drakensberg will change markedly with global warming. The presence or absence of Rhizobia in the soil will further complicate this. Those plants that have access to the elevated nitrogen levels as a result of these root nodule bacteria will have a distinct advantage over competitors growing without them.

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Abbreviations

CO_2, Carbon Dioxide
A, net rate of CO_2 fixation / assimilation
O_2, Oxygen
N_2, N, Nitrogen
C_a, ambient partial pressure of CO_2
C_i, intercellular partial pressure of CO_2
RSR, root:shoot ratio
RuBP, ribulose 1,5-bisphoshate
Rubisco, RuBP carboxylase
P_i, orthophosphate
$\mu l l^{-1}$, micro litres per litre = ppm = parts per million = µBar = microbars
FACE, Free Air Carbon dioxide Enrichment

CHAPTER 1

Introduction and Literature Review

1.1 Rising carbon dioxide levels

Due to the burning of fossil fuels and changes in landuse world wide, the atmospheric carbon dioxide concentration has increased during the past 100 years at an unprecedented rate (EAMUS and JARVIS 1989; ANTONIO and VITOUSEK 1992; SHLESINGER 1993; MITCHELL *et al.* 1995). The nominal atmospheric concentration of carbon dioxide is now *c.* 350 $\mu l l^{-1}$ and projections by atmospheric scientists suggest that this concentration will increase to approximately 700 $\mu l l^{-1}$ by the mid-21st century (BAZZAZ and WILLIAMS 1991; LEISHMAN *et al.* 1992; BAZZAZ and MIAO 1993). Increasing levels of CO_2 and other greenhouse gases are likely to result in significant changes to the earth's climate by the middle of the next century. One consequence of these large-scale climate changes will be a change in the distribution of many plant species. These shifts will have important consequences for land use patterns and feedback into the global climate (COLEMAN and BAZZAZ 1992; LEISHMAN *et al.* 1992; LINDROTH *et al.* 1993; SCHLESINGER 1993). Furthermore, global temperatures are predicted to increase in concert with increasing concentrations of CO_2 and other greenhouse gases (STRAIN 1987). These temperature increases could be as much as 3-5° C (PATTERSON and FLINT 1980; BOLIN *et al.* 1986; SCHLESINGER 1993), and will have substantial direct and indirect effects on the biosphere.

There is an extensive literature documenting the evidence for this increase in mean global CO_2 concentration and temperature (LAMARCHE *et al.* 1984; BOLIN *et al.* 1986; FIFIELD 1988; SCHNEIDER 1989; RAWSON 1992) from the mid-18th century through to the present day (EAMUS and JARVIS 1989; COLEMAN and BAZZAZ 1992). The evidence is based on ice core data (FIFIELD 1988), inferences from tree ring data (LAMARCHE *et al.* 1984) and climate modelling based on fossil fuel consumption and the CO_2 airborne fraction. The most recent evidence comes from measurements of global atmospheric CO_2 concentrations at different sites around the world (CRANE 1985, cited in EAMUS and JARVIS 1989).

An area of particular concern is the combined effects of increasing levels of CO_2 and temperature on the productivity (and thus nutrient status) of natural plant communities. Various authors have found a range of combined effects of elevated CO_2 and increased temperature (RAWSON 1992). RASTETTER *et al.* (1992) found a positive additive effect. IDSO *et al.* (1987, cited in RAWSON 1992)and JONES *et al.* (1993) found a less than additive effect, which indicates a decline in response to elevated CO_2 as temperature increases. This was species dependant. JONES and JONGEN (1996) found a negative effect. However, the variability of response even within any one species is disturbing (RAWSON 1992) for researchers, and further confuses any analysis.

1.2 The effects of elevated CO_2 on plants

The impact of rising CO_2 on crops and other herbaceous plants has been intensively studied. However, the direct and interactive effects of CO_2 and other environmental factors, such as nitrogen fixation, on tree species have received less attention (EAMUS and JARVIS 1989; BAZZAZ 1990; POLLEY 1997; PRIOR *et al.* 1997). Studies have mainly concentrated on northern Hemisphere plants and ecosystems: forests (BOLIN *et al.* 1986; BAZZAZ and WILLIAMS 1991; PRIOR *et al.* 1997); prairies (TIEDEMANN and KLEMMEDSON 1973; TIEDEMANN and KLEMMEDSON 1986); crops and tundra, while little work has been done on savanna and African grasslands (ALEXANDER 1989; BELSKY *et al.* 1989; ARNONE 1996; POLLEY 1997). Of the few studies that have been conducted on grasslands/savannas, most were neither large enough nor continued long enough to fully accommodate species and genetic change, shifts in soil properties, and other changes that must be understood to predict CO_2 effects on natural ecosystems (POLLEY 1997).

The growth response of plant species to enriched CO_2 is highly variable. Reviewed data for 250 species individually grown at ambient and elevated CO_2 revealed that plant biomass accumulation increased on average by 42% but the response ranged from -58% to +468% (POORTER *et al.*1996; cited in ROUMET and ROY 1996). Species belonging to the same genera did not respond more uniformly than species from different genera. Ecological criteria (C, S R plant strategies, GRIME 1977) did not reveal a homogenous response; only a few of the competitive species responded strongly (HUNT 1991). Physiological criteria have been used more widely; C_3 species are more responsive than C_4 or CAM species, fast growing species respond more than slow growing species, and

nitrogen-fixing species were also more responsive (POORTER 1993; POORTER 1996; ROUMET and ROY 1996). From a biochemical point of view, obligate CAM species could respond similarly to C_4 species if phosphoenolpyruvate carboxylase (PEP) were saturated at close to the current atmospheric CO_2 concentration. Nonetheless, facultative CAM species might respond more to elevated atmospheric CO_2 concentrations (DRENNAN and NOBEL 2000).

Experimentally, increased CO_2 levels increased net photosynthesis (BAZZAZ 1990; COLEMAN and BAZZAZ 1992; POORTER 1993; LETHIEC and DIXON 1996; MJWARA *et al.* 1996; SCHAFFER *et al.* 1996), water use efficiency (BOLIN *et al.* 1986; COLEMAN and BAZZAZ 1992; LETHIEC and DIXON 1996), the occurrence of symbiosis (HATTON and SMART 1984; NORBY 1987), reproductive potential, rooting (SCHAFFER *et al.* 1996), branching, tiller production, and growth (BAZZAZ 1990; GARBUTT *et al.* 1990; POORTER 1993; MJWARA *et al.* 1996; DRENNAN and NOBEL 2000;). Stomatal conductance (GARBUTT *et al.* 1990; TYREE and ALEXANDER 1993; RODEN and BALL 1996a; 1996b) and the concentration of nitrogen in plant parts (STRAIN 1987; GARBUTT *et al.* 1990; MJWARA *et al.* 1996; NORBY 1996) usually decline as CO_2 concentration increases. Increased photosynthesis from elevated CO_2 is, however, small in comparison to photosynthetic and growth responses to additions from other resources such as light, nutrients and water (BAZZAZ 1990; BAZZAZ and MCCONNAUGHAY 1992).

LINDROTH *et al.* (1993) found that trees responded to elevated atmospheric CO_2 with respect to every growth parameter measured, although responses varied among species. Dry matter production (total plant growth) ratios of elevated-to-ambient relative growth rates and growth rates increased. Total leaf mass and leaf mass per unit area increased in aspen, and oak, under elevated CO_2. However, neither parameter was significantly altered in maple trees. Increased CO_2 often increases root growth, and may thereby increase the access of woody seedlings to soil water both within and below the rooting zone of neighbouring plants (BREMER *et al.* 1996; POLLEY *et al.* 1996).

Increased plant water use efficiency (WUE) is often observed in plants grown in elevated CO_2. Many workers have ascribed these increases in WUE to greater photosynthetic rates (associated with greater CO_2 availability) and lower evaporative flux density (resulting from decreased stomatal conductance), or a combination of the two (ROGERS and DAHLMAN

1993; TYREE and ALEXANDER 1993; JONES and JONGEN 1996; KREMER *et al.* 1996; POLLEY 1997; DRENNAN and NOBEL 2000). While the biophysical mechanism by which stomatal conductance is decreased is still unclear, reductions of 30 - 50% are commonly found in herbaceous plants (KREMER *et al.* 1996; POLLEY 1997). Transpiration is reduced due to a lower stomatal conductance (POORTER 1993; TYREE and ALEXANDER 1993; JONES and JONGEN 1996; KREMER *et al.* 1996). The effect of elevated CO_2 on transpiration ranges from negligible to a nearly 70% decrease, depending on the species (BREMER *et al.* 1996). However, several workers (GARBUTT *et al.* 1990; ELLSWORTH *et al.* 1995; JONES and JONGEN 1996) have suggested that if plants grow larger and produce more leaf area, the increase in WUE could be offset, resulting in unchanged or even increased water use. Results did not confirm this though, as their plants used less water under elevated CO_2, which caused stomatal closure, but did not increase leaf area. Also, where the reduction in conductance is not offset by an increase in leaf area, established plants might delay dehydration by depleting soil water more slowly (POLLEY *et al.* 1996). This may benefit individually grown plants or species monocultures, but not seedlings in highly competitive environments where water saved by more slowly transpiring seedlings may be used by neighbouring plants, or lost to evaporation. Also, in wet years soil water does not limit productivity and conservation of soil water is of no advantage to plants (BREMER *et al.* 1996). These improvements in plant water relations are often the primary benefits to plants in which photosynthesis is insensitive to CO_2 (OWENSBY *et al.* 1993, cited in POLLEY 1997). Plant growth response is, however, almost always dependant on the edaphic conditions (i.e. soil water and nutrient supply). The greater the availability of both, the greater the growth enhancement with CO_2 (BAZZAZ 1990; MUELLER-DOMBOIS 1992). Moreover, adjustments in the distribution of the nutrient pool within the plant or in the metabolic requirements could lower nutrient demand, (i.e. increased nutrient use efficiency). For example, if the efficiency of Rubisco is higher under elevated CO_2, less N would be needed per unit of dry matter increment (NORBY *et al.* 1986).

1.3 Ecosystem responses to elevated CO_2

Elevated CO_2 has the potential to substantially alter competitive processes and change community composition (STRAIN 1987; COLEMAN and BAZZAZ 1992; MITCHELL *et al.* 1995; REEKIE 1996). Such changes in community composition could have important consequences by influencing the whole ecosystem response to CO_2. This will have

economic ramifications, which alter the equilibrium between unimportant and desirable species, and could affect herbage quality as well as biodiversity.

Ecosystem response to climate will not be determined by plant response alone. Elevated CO_2 and climate change may shift the balance between decomposition and production, altering nutrient supply and the accumulation of plant residues and soil organic matter (HUNT *et al.* 1996). Major responses under elevated CO_2, which affect the direct environment, include; massive starch accumulation, increased fine root production, (MJWARA *et al.* 1996; NORBY 1996) and a doubling of CO_2 evolution from the soil (KÖRNER and ARNONE 1992; LUO and MOONEY 1995). The deleterious levels of starch production in leaves in top canopies under elevated CO_2 may cause alterations in dominance relations and canopy / plant architecture in plant communities as CO_2 rises (BAZZAZ 1990; KÖRNER and ARNONE 1992). Stimulated rhizosphere activity is accompanied by an increased loss of soil carbon and increased mineral nutrient leaching (KÖRNER and ARNONE 1992). This may lead to a long-lasting depletion of mineral nutrients if unbound ions are leached from the rhizosphere. Further responses include greater carbon sequestering by terrestrial ecosystems, increasing amounts of leaf area per unit of land area, reduced water consumption and greater efficiency of nutrient capture (KÖRNER and ARNONE 1992; JOHNSON *et al.*1993; LUO and MOONEY 1995). With increasing concentrations of CO_2, NORBY (1996) found a significant increase in the percentage of mycorrhizal roots. This did not appear to be a short-term response, as the plants were grown for four years, and could therefore persist and shape the overall response of an ecosystem to elevated CO_2. Also, if temperature and moisture change in conjunction with elevated CO_2, it becomes more difficult to predict the effects on decomposition (HUNT *et al.* 1996). At temperatures above a species' temperature optimum, further increases in temperature may depress primary production while stimulating decomposition. It is therefore not obvious how elevated CO_2 and climate change will affect soil organic matter quality and quantity, or the degree of nutrient limitation (HUNT *et al.* 1996).

Low soil water is an important constraint on grassland invasion by woody species. There is limited evidence that rising CO_2 will increase osmotic adjustment, and thereby increase the tolerance of some plants to dehydration (POLLEY *et al.* 1996). However, improved WUE of plants under elevated CO_2 could lead to longer periods of an increased soil moisture content, causing anaerobic soil spots, which could result in a considerable loss of

available mineral nitrogen (HARTWIG *et al.* 1996). Under field conditions, nitrogen is almost always available to legumes. The extent of competition for soil-available nitrogen by associated non-legumes is one factor that determines the proportion of nitrogen derived from nitrogen-fixation (HARTWIG *et al.* 1996). This proportion will increase if available nitrogen is decreased by any mechanism. Symbiotic nitrogen-fixation would be able to account for an increased nitrogen sink of a whole ecosystem and this would buffer against a possible imbalance between carbon and nitrogen cycles which may occur under elevated CO_2 (HARTWIG *et al.* 1996). Symbiotic nitrogen fixation would then enable increased carbon sequestration into an entire ecosystem under elevated CO_2.

Under elevated atmospheric CO_2 on the global scale, grasslands and prairies might be better able to withstand periods of drought than they do under current ambient CO_2 levels. A CO_2-induced decrease in stomatal conductance causing a reduction in transpiration ultimately might reduce plant water use. This reduction in water usage, which would conserve soil water, might prolong plant vigour during periodic drought, as well as affect water balance, carbon balance and net productivity of grasslands and prairies (BREMER *et al.* 1996).

1.4 Leguminous trees and nitrogen fixation

The bacterial genus *Rhizobium* is responsible for nodule initiation and development and *R. leguminosarum* is said to be most prevalent in infecting the roots of legumes such as acacias (STEWART 1966). Leguminous trees are a valuable component in natural plant communities, just as legumes are important in agriculture. They are used for timber, firewood and in land reclamation serving as a barrier against soil erosion. Much of the natural bush in southern Africa is to a large degree dominated by *Acacia* species. Many of these and other leguminous trees are nodulated and can increase the total amount of nitrogen present in the system through nitrogen fixation (STEWART 1966; LANGKAMP *et al.* 1982; VAN KESSEL *et al.* 1983; HARTWIG *et al.* 1996). Because of their nitrogen fixing capability these plants may have an ecological advantage over other plants on nutrient-depleted soils (LANGKAMP *et al.* 1984). Legume/bacterial symbiosis is significantly increased by elevated CO_2 levels, this appears to be mainly due the larger biomass of these plants (ROGERS and DAHLMAN 1993). They are able to extract nutrients from a relatively poor soil, and return these via litter, thereby increasing the nutrient status of the

soils considerably (HATTON and SMART 1984). The extent to which *Acacia holosericea* assists in the re-establishment of a nitrogen-nutrient stock has been estimated to be 12 ± 4 kg N ha $^{-1}$ year $^{-1}$ (LANGKAMP *et al.* 1979). However, BARNET *et al.* (1985) contend that there is very little direct evidence to support the assumption that re-vegetating disturbed areas with legumes will ensure an adequate supply of nitrogen for the area. Seasonal changes in nodulation and nodule specific activity (lower in the dry season) produce marked variation in fixation rates (LANGKAMP *et al.* 1982; BARNET *et al.* 1985).

It is possible that the improved status of the soil beneath the canopies of many *Acacia* species, and other legumes (TIEDEMANN and KLEMMEDSON 1973; LANGKAMP *et al.* 1982;TIEDEMANN and KLEMMEDSON 1986; ALEXANDER 1989; BELSKY *et al.* 1989) is due to the nutrient composition of the leaves and seed pods, the improved quality of which is, in part, due to the nitrogen fixing ability of these trees. The forms of nitrogen, which are readily available for plant absorbtion, are NH_4^+ and NO_3^- in the labile pool. The amount of N in the labile pool is determined by the balance of several fluxes, including plant uptake, mineralization of soil organic N, immobilisation of labile N, deposition, fixation and denitrification, volatilisation and leaching (LUO and MOONEY 1995). Increasing levels of carbon in terrestrial ecosystems under elevated CO_2 conditions may directly alter plant uptake, fixation and mineralization/ immobilisation, leading to changes in ecosystem nitrogen dynamics (LUO and MOONEY 1995).

1.5 The effects of elevated temperature and nitrogen on plant growth

The projected doubling of the CO_2 concentration by the middle of the next century will increase carbon availability, but the implications for plant nitrogen acquisition are still unclear (BASSIRIRAD *et al.* 1996; WAND *et al.* 1996). Although the growth of tree seedlings is increased by CO_2 enrichment, even under nitrogen-deficient conditions over the short term (NORBY 1996), it is hypothesised that increases in nitrogen availability may be necessary to sustain enhanced growth rates for many seasons (NORBY 1987; EAMUS and JARVIS 1989; BAZZAZ 1990; BASSIRIRAD *et al.* 1995; LUO and MOONEY 1995; MIGLIETTA *et al.* 1996). Nitrogen-fixing trees are important for providing a stable, long-term source of nitrogen in many silvicultural systems. In unmanaged ecosystems, nitrogen-fixing trees not only increase nitrogen accretion rates, but may also accelerate mineralization rates of other nutrients (NORBY 1987). Plants grown at elevated CO_2 levels may have more

carbohydrates available for the support of mycorrhizal or N-fixing bacteria, improving their nutrient status (BAZZAZ and MCCONNAUGHHAY 1992). Furthermore, elevated CO_2 often reduces plant nitrogen concentration (GARBUTT *et al.* 1990; BASSIRIRAD *et al.* 1996; CAMPBELL and HART 1996; MJWARA *et al.* 1996; WILSEY 1996). Others (LUO and MOONEY 1995; VOLIN and REICH 1996) describe how photosynthetic rates, growth and leaf area expansion were significantly greater for plants grown with high nitrogen compared to those grown in low nitrogen. MITCHELL *et al.* (1995) found that nitrogen supply markedly influenced respiration rate. Both nitrogen and CO_2 increases and the interaction of these two factors significantly increased longleaf pine respiration rates. However, nitrogen supply had a greater effect on both respiration and needle growth than did atmospheric CO_2 concentration.

RASTETTER *et al.* (1992) found that, under increased temperatures, a major cause of increased carbon storage in tundra and forest ecosystems was the internal redistribution of nitrogen. At higher temperatures the rates of decomposition and nitrogen mineralised increased. This increase in mineralization increased nitrogen availability to vegetation and stimulated productivity, the net effect being a large shift in nitrogen from the soils to the vegetation. Large increases in temperature may cause severely reduced growth or increased mortality by disrupting membrane integrity and enzyme function (OUGHAM and HOWARTH 1988). Different temperature responses in component species from the same communities are a result of each species having its own optimum temperature regime for growth leading to a different seasonal pattern of growth for each component species (COLEMAN and BAZZAZ 1992; STEWART and POTVIN 1996). The additional effect of elevated CO_2 in the system complicates the results further. Enrichment with CO_2 can moderate adverse effects of temperature extremes (ROGERS and DAHLMAN 1993; BOWES *et al.* 1996). CAMPBELL and HART (1996) found that plant growth was stimulated by elevated CO_2 at both low (18/13°C, day/night) and high temperatures (28/23°C), but more so at the higher temperatures. However, they found marked differences in the effect of elevated CO_2 when the plants were grown with companion species (competitors).

Higher global temperatures are an important consideration in the rising CO_2 debate because of the interactive effects on photosynthesis. A rise in temperature lowers the ratio of $[CO_2]/[O_2]$ in solution, shifts the specificity of Rubisco towards oxygenase, enhances photorespiration and dark respiration, and increases the sink response relative to the

source. Thus positive effects of CO_2 enrichment are potentially greater as the temperature rises, but the exact effects may be complicated (ROGERS and DAHLMAN 1993; BOWES *et al.* 1996; HUNT *et al.* 1996). CAMPBELL and HART (1996) found that the effect of CO_2 was strongly determined by the prevailing conditions of temperature. Temperature optima of *Trifolium repens* and two grasses (C_3/C_4) were vastly different. A seasonal switching in competition between species with high and low temperature optima may act to reduce the competitive dominance exerted by any one component of the community in response to elevated CO_2. However it is suggested that the greatest change in competition in response to CO_2 is likely to occur during *warmer* periods, favouring those species competing most effectively at that time. C_4 grass species generally showed lower responses to CO_2. There is already a strong seasonal switching from temperate (C_3) grass dominance in spring to greater clover (C_3) dominance in summer in warm temperate grasslands (CAMPBELL and HART 1996; STEWART and POTVIN 1996). Changes in competitive interactions with future increases in atmospheric CO_2 may therefore have the effect of accentuating this existing seasonal trend. The most aggressive competitors will be those with more competitive attributes, and the weaker competitors those classified as closer to the *R* or *S* strategies (GRIME 1977; CAMPBELL and HART 1996).

Growth in elevated CO_2 can ameliorate the effects of stress events for plants (ROGERS and DAHLMAN 1993; VOLIN and REICH 1996). Elevated CO_2 can compensate for water or nutrient stress, as well as for low light in some species (LEISHMAN *et al.* 1992; ROGERS and DAHLMAN 1993; TYREE and ALEXANDER 1993; RODEN and BALL 1996a; 1996b), and ozone (O_3) poisoning in others (ROGERS and DAHLMAN 1993; VOLIN and REICH 1996). However, the predicted ecological effects are unclear. Elevated CO_2 may allow plants to survive at lower nutrient levels, but the plant growth response to increased access to nutrients is greater under elevated CO_2 (BAZZAZ 1990; WONG *et al.* 1992). Grasses have also been shown to be effective competitors for water and nutrients. Competition for nitrogen was one of the factors responsible for poor pine seedling growth in the presence of alien grasses (ANTONIO and VITOUSEK 1992). Consequently, elevated CO_2 could be an advantage to plants that can obtain extra nitrogen by fixation (LEISHMAN *et al.* 1992; POORTER 1992; HARTWIG *et al.* 1996; STEWART and POTVIN 1996).

1.6 Competition between C_3 and C_4 plants in elevated CO_2

Differential responses of species to CO_2 fertilization will alter community structure and function (STRAIN 1987; BAZZAZ and MCCONNAUGHAY 1992; COLEMAN and BAZZAZ 1992; JOHNSON *et al.* 1993; JONES and JONGEN 1996; RODEN and BALL 1996a; 1996b; STEWART and POTVIN 1996). In vegetation where floristic balance is currently maintained by interspecific competition, increases in vigour of a competitor can be expected to affect the other species detrimentally (BAZZAZ and MCCONNAUGHAY 1992; JOHNSON *et al.* 1993; REEKIE 1996). As a group, C_4 plants have an average photosynthetic rate around 50% higher than C_3 plants. In terms of carbon uptake in photosynthesis and growth, C_4 plants have been categorised as 'efficient' in contrast to 'non-efficient' C_3 plants (JOHNSON *et al.* 1993; ROGERS and DAHLMAN 1993). However, the photosynthetic rates of C_3 plants show the greatest sensitivity to changing CO_2 levels (JOHNSON *et al.* 1993; ROGERS and DAHLMAN 1993). As a result, plants that photosynthesize by the C_3 pathway are expected to outgrow C_4 competitors as CO_2 continues to increase (PATTERSON and FLINT 1980; MARKS and STRAIN 1989; HUNT *et al.* 1996). Carbon dioxide is assimilated by the enzyme Rubisco in leaf mesophyll cells of plants with the C_3 photosynthetic pathway. Virtually all trees and shrubs, and most of the 'cool season' grasses are C_3 plants. Rubisco reacts with oxygen in air, resulting in a loss of CO_2 in a process called photorespiration. Under current atmospheric conditions and moderate leaf temperatures, 30 - 50% of the total amount of carbon assimilated in C_3 photosynthesis is lost to photorespiration (LONG and HUTCHIN 1991, cited in POLLEY 1997). That proportion decreases as CO_2 concentration rises and temperature declines. In plants which photosynthesize by the C_4 pathway (most temperate, tropical grasses) carboxylation by Rubisco occurs in the leaf bundle sheath cells using CO_2 that was initially assimilated by the enzyme phosphoenol pyruvate (PEP) carboxylase. Because PEP carboxylase does not react with O_2 and C_4 plants maintain high CO_2 concentrations in bundle sheath cells, photorespiration is negligible in these species (POLLEY 1992). Net photosynthesis is higher in C_4 than C_3 plants at low CO_2 concentrations, but C_4 photosynthesis usually becomes CO_2 saturated at comparably low concentrations (POLLEY 1997).

Higher temperatures increase photorespiration in C_3 plants. By reducing photorespiration, higher CO_2 increases the temperature optimum for CO_2 uptake and the maximum temperature at which positive photosynthesis can occur in C_3 plants. CAMPBELL and HART

(1996) found that subtropical C_4 grasses showed no positive responses to CO_2 at high or low temperature, while the dry mass of C_3 grasses was increased by CO_2 at both temperatures. C_4 plants may, however, be favoured in warmer, drier environments (COLEMAN and BAZZAZ 1992; CAMPBELL and HART 1996) regardless of the CO_2 concentration, although PATTERSON (1986) found the opposite effect. Studies of C_3 photosynthesis and plant growth have demonstrated that plants usually respond relatively more to an increase in CO_2 concentration when temperatures are high (POLLEY 1997). However, different results have been encountered under different experimental conditions (BAZZAZ and MCCONNAUGHAY 1992). While elevated CO_2 may influence competitive interactions, other resources such as nutrients, light, temperature and nitrogen fixation will influence these interactions to a greater degree.

If atmospheric CO_2 remains high, increased water-use-efficiency should allow more plant growth during episodes of drought and the expansion of species ranges into drier habitats (BAZZAZ 1990). However, MARKS and STRAIN (1989) found that the competitive outcome between aster and broomsedge was not altered by water availability, but was changed by CO_2 enrichment. Relative species contribution to total above ground production changed under elevated CO_2 from 50% each, to favour aster at 75% of total above ground production. The responses of individually grown plants vary markedly when these plants are grown together in mixtures. Knowledge of the relative responsiveness of each species to elevated CO_2 will be crucial to attempts to model the effects of CO_2 on community composition (REEKIE 1996).

Success in recolonizing disturbed land is mainly a function of seedling competition and the success of legumes could reside with factors other than nitrogen fixation. These include seed number, seed size, hardseededness and speed of seedling establishment (LANGKAMP *et al.* 1982). Larger seeded species are better able to establish in short herbaceous turf, which provides a steep vertical gradient of light, while an initially larger seedling could project its leaves into a better light climate and so be at an advantage (LEISHMAN *et al.* 1992). It is likely that elevated CO_2 will have an impact on species capabilities to establish seedlings under different circumstances.

The photosynthetic advantage of C_4 plants at low CO_2 concentrations is achieved at the expense of an additional energy requirement. Because this energy comes from light,

maximum photosynthesis per unit of absorbed light is lower in C_4 plants than in C_3 species, in which photorespiration is inhibited by high CO_2 concentration. Elevated CO_2 has a particularly beneficial effect on photosynthesis at low light levels for C_3, but not C_4 plants (BAZZAZ 1990; LEISHMAN *et al.* 1992; POLLEY 1997). This could bring about a substantial change in existing vegetation dynamics, with greatly increased rates of establishment of many species of C_3 seedlings under closed vegetation. BAZZAZ and WILLIAMS (1991) suggest that in forested conditions the CO_2 levels are likely to vary along a height gradient with the greatest concentration of CO_2 being near the forest floor. Thus seedlings, saplings and mature trees may experience different CO_2 environments and show different responses in photosynthesis, growth and water use.

Grasses are good competitors against herbaceous and woody species (ANTONIO and VITOUSEK 1992). The establishment of large seeded and woody perennials has been found to be limited in the presence of dense grasses or grass litter. Thus the invasion of grasslands by other perennial species often requires soil disturbance (digging, burrowing and stomping by animals, or the formation of any number of different types of 'mounds')(COX and GAKAHU 1985; MIDGLEY and MUSIL 1990; ANTONIO and VITOUSEK 1992; DEAN and YEATON 1993a; 1993b; RUGGIERO and FAY 1994). Rapidly growing grasses can reduce light at the soil surface and thereby reduce the photosynthetic ability of competitors. Mechanistic understanding is needed of (i) how CO_2 may alter competition for resources; (ii) how competition processes are influenced by the functional characteristics of the organisms making up the community; and (iii) how other environmental variables will alter the effect of CO_2 on competitive interactions.

1.6.1 Ecological theory

Since increases in CO_2 can represent an increase in resource availability, the community and ecosystem can be regarded in terms of ecological theory with respect to this resource. From this theory, it is predicted that plants from productive habitats will be the most rapid to increase competitive effects in response to increases in resource availability, i.e. a more aggressive response can be expected from competitive plant species (GRIME 1977; GORDON and RICE 1993; CAMPBELL and HART 1996). As a result, the competitive effects of fast growing plants from productive habitats should increase more with elevated CO_2 than the competitive effects of plants with lesser competitive ability. Consideration needs to be taken of the differences between the growth rates of C_3 and C_4 plants. Many workers

(PATTERSON and FLINT 1980; BAZZAZ 1990; COLEMAN and BAZZAZ 1992; CURE and ACOCK 1996) have found that C_3 plants can be more responsive to increased CO_2 levels than C_4 plants.

Thus we might predict that in medium-to-high fertility conditions fast growing C_3 species from fertile habitats would develop greater short-term competitive effects at high CO_2 than would C_4 species or species of lesser competitive ability from infertile habitats (CAMPBELL and HART 1996). Increases in temperature will also shift the competitive outcome. CAMPBELL and HART (1996) observed that at lower temperatures C_3 grasses exerted the greatest competitive suppression on *Trifolium repens* (C_3), and at high temperatures the C_4 grasses were the most aggressive. Secondly, the most aggressive competitors against *Trifolium repens* with elevated CO_2 were those grasses with more competitive attributes, whereas the weaker competitors were generally grasses classified as closer to the *R* or *S* strategies (GRIME 1977) in ecological theory. Results were therefore consistent with predictions of plant strategy/ecological theory, and highly variable depending on the combination of factors at work.

1.7 The effects of elevated CO_2 on other biological processes

Biological nitrogen fixation by symbiotic micro-organisms, free living bacteria and cyanobacteria has fixed most nitrogen in vegetation and soils (LUO and MOONEY 1995). The energy supply driving this fixation is entirely from the oxidation of photosynthetically fixed carbohydrates. The amount of nitrogen available for mineralization within an ecosystem is determined by the availability of the reduced carbon energy sources from plants. Thus as CO_2 consistently increases the sugar and starch content of vegetation, it is likely that the nitrogen content in an ecosystem will increase to match this increased photosynthetic potential in the long term (LUO and MOONEY 1995). If plant nitrogen content declines as predicted by PARRISH and BAZZAZ (1985) and GARBUTT *et al.* (1990), it may have important consequences for plant herbivory, decomposition and plant-plant interactions. Enhanced plant growth invariably leads to low nutrient status within the plant, due to a dilution of minerals by higher concentrations of carbon (MJWARA *et al.* 1996). Because leaf and seed biomass generally increases under elevated CO_2, the amount of nitrogen as a percentage of total biomass decreases, dependant on species. These changes in leaf and seed nitrogen content caused by elevated CO_2 may, in the long term,

be more important in altering community structure than the more obvious differences seen in other characteristics (PARRISH and BAZZAZ 1985; STRAIN 1987; GARBUTT *et al.* 1990; WILSEY 1996). Herbivorous animals may be negatively affected if biomass production remains unchanged and percentage nitrogen decreases under elevated CO_2 conditions. Carbon:Nitrogen ratios in plant parts are predicted to increase and thereby decrease the nutritional value of foliage. Herbivorous animals may be forced to consume larger amounts of vegetation in order to meet their nutritional requirements, the ramifications of which are many and complex.

Growth of early instar larvae (NORBY 1996) and *Junonia* larvae (FAJER *et al.* 1989 cited in GARBUTT *et al.* 1990; and in ROGERS and DAHLMAN 1993) was significantly reduced when fed leaves of plants grown in elevated CO_2 because of a lower concentration of nitrogen in these leaves. Older larvae were not affected by the lower nitrogen concentration because of a compensatory increase in nitrogen utilization efficiency (NORBY 1996).

WILSEY (1996) found no large increases in above ground biomass in response to elevated CO_2. ARNONE *et al.* (1995) found no major differences in leaf nutrient quality between elevated and ambient CO_2 treatments. Since this is the portion of the plant consumed by grazing mammals, the quantity of the food available to them would be largely unaffected by elevated CO_2. However, plant quality as indexed by leaf percentage nitrogen was substantially decreased in response to elevated CO_2 (ROGERS and DAHLMAN 1993; WILSEY 1996). Therefore the same amount of food would be available, but of a lower quality.

1.8 Plant acclimation to elevated CO_2 levels

Despite the consistent evidence from short-term experiments for the direct stimulation in growth by increasing CO_2 there has been some reluctance to accept that prolonged growth in elevated CO_2 stimulates yield under normal conditions. This is largely because it has been assumed that photosynthesis is often limited by other environmental variables such as temperature, water and nutrient availability (JONES and JONGEN 1996). There is also much evidence, although very few field observations, that the initial CO_2 stimulation of photosynthesis is not maintained, and that down regulation occurs. The present consensus is that this is a very complex situation and involves the interaction of many variables (e.g.

temperature, nutrient and water status, altitude, photosynthetic pathway and potential rooting volume) (JOHNSON *et al.* 1993; MIDGLEY *et al.* 1995).

Possible explanations proposed for growth and photosynthetic acclimation to elevated CO_2 are; decreases in the amount and activation of ribulose bisphosphate carboxylase, chloroplast damage due to excessive accumulation of carbohydrates; and end product inhibition because of insufficient sinks in the plant (SAGE *et al.* 1989; ARP 1991; MIDGLEY *et al.* 1995; MIGLIETTA *et al.* 1996; VOLIN and REICH 1996). A further possibility is the transient nature of growth stimulation, especially in fast-growing plants. Relative growth rate (RGR) can decrease with time (POORTER 1993). As plants grow taller, more biomass is invested in support tissue, and these plants then suffer more from self-shading (POORTER 1993). Lack of response may be due to negative feedback, and the inability of the plant to efficiently translocate an excess of accumulated carbohydrates (SAGE *et al.* 1989; TYREE and ALEXANDER 1993). Feedback inhibition from the source occurs when the ability of the source to supply assimilates exceeds sink capacity (SCHAFFER *et al.* 1996). Such limitations are expected to occur most widely in nitrogen-limited plants as these are unable to generate new sinks for carbohydrates in response to the increased photosynthate supply (MIGLIETTA *et al.* 1996). The possible homeostatic nature of both photosynthesis and growth of many species, regardless of nitrogen availability, may also keep photosynthesis and growth within a given range (TISSUE and OECHEL 1987).

Root growth may be enhanced more than shoot growth under elevated CO_2. It is possible that initial widespread reports of photosynthetic acclimation under CO_2 enrichment could have been due to limited root expansion in small pot volumes (DRAKE and LEADLEY 1991; STOCK and MIDGLEY 1995). SCHAFFER *et al.* (1996) found that CO_2 enrichment may have compensated for the effects of root restriction on plant growth, since there was no effect of root chamber size on plant dry weight at elevated CO_2. LUO and MOONEY (1995) and VOLIN and REICH (1996) found that the plants, grown in elevated CO_2 and high nitrogen, had greater root mass and higher photosynthetic rates than those grown in elevated CO_2 and low levels of nitrogen. They suggested that if pot size eliminated the CO_2 enhancement of photosynthesis, it should have had a greater impact on plants grown in high nitrogen, because of their substantially larger root systems. This pattern was not found (VOLIN and REICH 1996). BERNSTON *et al.* (1993, cited in ARNONE 1996) showed that increasing the nutrient additions could eliminate the effects of small pot size.

1.9 Photosynthetic Response Curves (or A/C_i)

Rubisco (ribulose bisphosphate carboxylase-oxygenase) is the enzyme that initiates both the photosynthetic carbon reduction cycle (PCR), and the photorespiratory carbon oxidation (PCO) cycle, and is a major component regulating CO_2 assimilation in C_3 species. The kinetics of Rubisco have been used to model A/C_i curves. The net CO_2 assimilation (A) vs. C_i response curve has been widely used to determine the long-term CO_2 enrichment on the short term response of photosynthesis to intercellular CO_2 (C_i). The initial linear phase of the A/C_i curve is a measure of carboxylation efficiency because photosynthesis is limited by the amount of active Rubisco. A reduction in the initial slope of the A/C_i response curve under elevated CO_2 can be interpreted as a decrease in Rubisco capacity (SAGE *et al.* 1989; SAGE 1994). The reduction in Rubisco capacity on exposure to high CO_2 has been reported and explained as a decrease in enzyme activation state or the amount of Rubisco or both, and may indicate greater nitrogen use efficiency. As a large proportion of leaf protein (nitrogen) of the C_3 plant is found in Rubisco, a decrease in plant nitrogen concentration is often indicative of a decrease in the amount of Rubisco.

This phase is followed at higher concentrations of CO_2 by an inflection above which A rises more gradually and is limited by the rate at which RuBP can be regenerated by the PCR cycle. During photosynthesis C_3 leaves maintain C_i at close to the inflection point such that Rubisco, and RuBP regenerating capacity are co-limiting. A doubling in the CO_2 concentration reduces stomatal limitations because C_i rises from about 245 to 490 $\mu l l^{-1}$ CO_2. The rise in C_i causes the initial 50% increase in photosynthesis so often reported and moves photosynthesis beyond the RuBP-limited region of the A/C_i curve (SAGE *et al.* 1989; SAGE 1994).

Elevated levels of CO_2 enhance photosynthesis by creating greater internal CO_2 concentrations, thereby increasing the activity of Rubisco and consequently carbohydrate production (KREMER *et al.* 1996). In C_3 plants exposed to long-term high CO_2, the rate of CO_2 assimilation at high CO_2 does not always increase but in many cases is unchanged or may decrease in comparison to plants grown at ambient CO_2 (TISSUE and OECHEL, 1987). An upward regulation of photosynthesis results in a net increase in CO_2 assimilation, and a downward regulation, a lower photosynthetic rate. Downward regulation

can be partly attributed to a decrease in Rubisco capacity (V_{cmax}) (SAGE 1994; HOGAN *et al.* 1996; TUBA *et al.* 1996). The decrease in Rubisco activity is often correlated with a decrease in leaf nitrogen concentration. The values of V_{cmax} do not differ between treatments when Rubisco activity is not affected by CO_2 treatment (SAGE 1994; HOGAN *et al.* 1996).

ELLSWORTH *et al.* (1995) found no significant difference in net assimilation (A_{net}) measured at a common CO_2 concentration at either 350 or 550 $\mu l l^{-1}$ CO_2 between foliage from FACE (Free Air Carbon dioxide Enrichment) trees compared to reference trees not exposed to elevated CO_2. The ratio of C_i to C_a at C_a = 350 $\mu l l^{-1}$ was marginally lower for ambient grown plants compared to plants grown under elevated CO_2, although stomatal conductance (g_s) did not differ significantly. SAGE (1994) also reported of a number of studies in field grown plants since 1986 in which long-term CO_2 exposure produced little change in the response of net CO_2 assimilation, indicating a lack of acclimation to elevated CO_2 in natural environments. Plant performance may not improve as a result of a range of secondary plant responses to stress or changes in resource levels. High CO_2 may cause extensive carbohydrate accumulation in leaves which can interfere with proper chloroplast function. Also, if CO_2 enrichment increases plant size relative to the supply of nutrients, the availability of nutrients for photosynthesis may decline, reducing photosynthetic capacity (SAGE *et al.* 1989; POORTER 1993; SAGE 1994).

Typically, in C_3 plants under light saturated conditions for photosynthesis, Rubisco capacity limits A at low C_i, thylakoid dependant RuBP regeneration is limiting at intermediate C_i, and P_i regeneration becomes limiting at elevated C_i (SAGE 1994). Increasing P_i regeneration while decreasing Rubisco capacity is the most effective response, as both photosynthetic capacity and resource use efficiency are increased. The best way to determine the effects of elevated CO_2 using A/C_i data would involve determination of the ratio of A from plants grown at elevated C_a to plants grown at normal C_a when both are measured at the same C_i. If photosynthetic acclimation to elevated CO_2 has occurred, this assimilation ratio will differ from one across a broad range of C_i values; a value greater than one indicating greater CO_2 assimilation under elevated CO_2, and a ratio less than one indicating down regulation under elevated CO_2.

1.10 Stomatal Conductance

An important procedure for assessing photosynthetic acclimation to elevated CO_2 content is the comparative analysis of CO_2 and water vapour exchange between the atmosphere and leaves of plants growing at varying concentrations of atmospheric CO_2. At low CO_2 and saturating light intensity, the capacity of Rubisco to carboxylate RuBP is limiting for photosynthesis and the slope of the initial response of A to C_i (often termed carboxylation efficiency) is directly dependant on Rubisco content (SAGE 1994). In addition to directly increasing the potential rate of RuBP consumed by Rubisco, increasing the CO_2 increases the rate of CO_2 fixation by reducing the RuBP oxygenation and photorespiration. Because O_2 competes with CO_2 for RuBP, photosynthesis that is limited by the thylakoid capacity for RuBP regeneration is sensitive to CO_2 and O_2 levels. At low C_i, this dependency is pronounced, but as C_i increases, CO_2 increasingly out-competes O_2 for RuBP and the CO_2 sensitivity of A declines. As a result, the slope of the A/C_i response curve progressively declines as C_i increases above 200 $\mu l l^{-1}$. However, because CO_2 does not effectively limit photorespiration until well above 1000 $\mu l l^{-1}$, A is enhanced by increasing C_i to at least 1000 $\mu l l^{-1}$.

The C_i/C_a ratio is a useful index of possible stomatal acclimation to long-term CO_2 exposure, which directly reflects the relationship between stomatal conductance and the biochemical capacity for CO_2 fixation. If stomata independently acclimate to changes in the CO_2 level under which the plants are grown, then C_i/C_a should change. A conservative pattern of acclimation would occur if stomata close relative to photosynthetic activity and reduce C_i/C_a. This could have great benefit if water is more limiting for growth than carbon. Alternatively, if carbon is limiting and water is not, plants may open stomates relative to photosynthesis to maximise CO_2 uptake, thereby increasing C_i/C_a (SAGE 1994).

In C_3 plants stomatal conductance is regulated to track A and maintain the intercellular CO_2 concentration 20 to 30% below the ambient CO_2 partial pressure, although the magnitude of the reduction in C_i relative to C_a is sensitive to evaporative demand (SAGE 1994). Stomata are directly sensitive to C_i, and increases in C_i are well known to reduce stomatal conductance (CURE and ACOCK 1996). The reduction in stomatal conductance and the associated increase in A resulting from CO_2 enrichment are the principal factors increasing water use efficiency at elevated C_a (EAMUS 1991; SAGE 1994).

Direct effects of elevated CO_2 on C_3 plants include reduced photosynthetic capacity but enhanced net photosynthetic rates (A) at the growth CO_2 concentration, increased apparent quantum yield, decreased leaf nitrogen concentration and decreased dark respiration rates (KUBISKE and PREGITZER 1996). C_4 plants generally show little adjustment in the A/C_i relationship as a result of growth in different CO_2 regimes, unless nutrient levels become deficient. A lack of response in C_4 plants is not surprising, given that A is largely CO_2 saturated at C_a above 400 $\mu l l^{-1}$. (See earlier *Competition between C_3 and C_4 plants in elevated CO_2*). However, it has been observed that the CO_2 photosynthetic response curves of various C_4 species indicate that photosynthesis is not always saturated at an ambient concentration of 600 µll-1 (POORTER 1993). It seems that in some species the C_4 pathway is not as tightly controlled as previously suggested, and therefore allows some response to CO_2 (POORTER 1993).

1.11 Possible effects of global warming in KwaZulu-Natal

As the CO_2 concentration world-wide changes, it is useful to predict how the vegetation will change, and in which successional direction. In this way future land use options can be considered. The most useful plants for a given scenario can be studied and planted in anticipation of their future growth advantage, especially in terms of land reclamation and rehabilitation (LANGKAMP *et al.* 1982; VAN KESSEL *et al.* 1983; BARNET *et al.* 1985). However, predicting the direction, much less the magnitude, of changes in plant function is difficult because there is a lack of understanding of the mechanisms that control overall plant response to CO_2 concentration (MITCHELL *et al.* 1995). The predicted increase in mean global temperatures is between 1.6 and 3.5°C to the year 2070 (IPCC 1990). The effects of global warming are expected to be greatest in the higher latitudes of both hemispheres and least in the tropics (MANABE and WETHERALD 1980; cited in LEISHMAN *et al.* 1992).

Carbon dioxide is the substrate for photosynthesis for all terrestrial higher plants, with C_3 plants growing in adequate light requiring 800-1000$\mu l l^{-1}$ for saturation of photosynthesis (JONES and JONGEN 1996). Carbon dioxide fertilization increases nodulation and nitrogen fixation (HATTON and SMART 1984; NORBY 1987) and enhances the establishment and growth of those C_3 trees that fix nitrogen. In this present study the effects of CO_2 fertilization and root inoculation was studied in two *Acacia* species. HATTON and SMART

(1984), found that acacias (*A. sieberana*) in Uganda were able to extract nutrients from a relatively poor soil and return these via the litter, thereby increasing the nutrient status of the soils. Similar results were found for *Acacia albida* in Sudan and Nigeria, and *Azadirachta indica* in northern Nigeria where they improved both the nutrient status and physical conditions of the soil considerably (HATTON and SMART 1984; ALEXANDER 1989). TIEDEMANN and KLEMMEDSON (1973; 1986) found that the level of total nitrogen was three times greater in the soil under mesquite trees than in non-mesquite soil. The yield of grasses growing under mesquite also increased.

In southern Africa, and Africa as a whole, various trees are in great demand for their firewood and medicinal properties. It would be useful to know if these trees prove to be those which benefit more than other species from increased CO_2 concentrations (VAN KESSEL *et al.* 1983). Concern exists about the encroachment of woody plants into the KwaZulu-Natal grasslands, particularly *Acacia* spp. which are both C_3 and potentially nitrogen-fixing plants and thus, may benefit from global warming. Nothing is known about the effects of cold temperatures in limiting the distribution of *Acacia* species at high elevations. Increased temperatures may enable some plants to grow at higher altitudes. With the potentially added benefit of enhanced growth under CO_2 enrichment, entirely new habitats for growth and reproduction will be available to these plants. If such a plant was fast-growing, useful for firewood, improved the soil nitrogen availability, and soil nutrient status and condition, (ALEXANDER 1989; BELSKY *et al.* 1989; TIEDEMANN and KLEMMEDSON 1973; TIEDEMANN and KLEMMEDSON 1986) as well as being helpful in reducing soil erosion, it would be most fortuitous. The improved condition of soil beneath these trees would also enhance grass growth and quality (whether a C_3 grass in the shady elevated CO_2 conditions, or a C_4 grass at elevated temperatures).

Temperature influences plant growth by affecting the biochemical processes controlling resource acquisition, growth and energy budgets. For example, warmer temperatures may stimulate plant growth and photosynthesis, but may simultaneously increase rates of transpiration increasing the probability that water will limit plant productivity (COLEMAN and BAZZAZ 1992). The predicted rise in global temperatures may affect the productivity and composition of plant communities because different species from the same community may exhibit very different responses to increasing temperatures. Many high altitude areas in the Drakensberg are at present almost completely bereft of trees, due to cold

temperatures, and harvesting for firewood by the local populations. A climate change as a result of a change in CO_2 and other greenhouse gas concentrations may therefore benefit future tree growth. If the predictions of atmosphere and climate changes are accurate, it would be useful to pre-empt it, and the subsequent vegetation change, in order to plan more effectively and beneficially for both the environment and people involved. It is important to keep in mind that the CO_2 problem, or rather the problem of a changing climate due to emissions of greenhouse gases into the atmosphere cannot be considered in isolation. It is one of many environmental problems that must be addressed, but in long-term perspective it is probably the most important one (BOLIN *et al.* 1986).

1.12 Objectives and Introduction to the Present Investigation

The main objectives of the present investigation were to test the effects of elevated carbon dioxide on the *Acacia/Rhizobium* symbiosis and on plant growth, and the effects of altitude related temperature differences, on two *Acacia* species from KwaZulu-Natal.

A series of experiments were initiated involving the establishment from seed and from transplanted seedlings and saplings of important *Acacia* species a) within greenhouse CO_2-enriched environments, b) within and above their elevational distribution, to answer the following questions:

1. a) How do plants grown in an elevated CO_2 environment compare to those grown at ambient concentrations of CO_2, with respect to growth parameters such as above ground biomass, root biomass, height and leaf area, and what effect does the presence of root nodules have on this comparison?
   b) What are the effects of these two treatments (elevated/ambient CO_2 and presence/absence of *Rhizobia* sp.) on photosynthetic parameters like the A/C_i response and stomatal conductance?
   c) What are the effects of these two treatments on nitrogen concentration in acacias?
2. How does transplanting *Acacia* to the centre, upper elevational limits and above the limits of their present distribution affect survival and growth?
3. How do C_3 and C_4 plants competitively grown in an elevated CO_2 environment compare to those grown at ambient concentrations of CO_2, with respect to height and biomass acquisition, and what are the implications for community structure?

The direct assessment of the impact of rising CO_2 is very difficult, because of the long term nature of CO_2 effects and the myriad of potential interactions between CO_2 and other environmental factors that can influence physiological and ecological relationships (NORBY 1996). The best approach is to use FACE or even larger scale enclosures, but these are very expensive to erect and costly to run, due to the sophisticated equipment involved (TIBBITTS and LANGHANS 1993) and the large volumes of CO_2 expelled each day. As a result, plants were grown in simple chambers under greenhouse conditions (see Chapter 2 for more details).

CHAPTER 2

Plant growth in response to CO_2 enrichment with two different *Rhizobium* inoculation treatments

2.1 Introduction

Many studies have shown that on a short-term basis, increased photosynthetic activity results in increased biomass acquisition when plants are grown at elevated CO_2 concentrations (HOGAN *et al.* 1996). It is uncertain whether these effects persist as trees age. Several factors may interact to determine whether this increase in growth rate persists, or if down-regulation occurs over time. These factors include; nutrient availability, temperature, the size of the pot, a combination of root restriction affecting source-sink relationships, and the soil volume determining the total amount of available nutrients (THOMAS and STRAIN 1991; HOGAN *et al.* 1996). It is therefore difficult to extrapolate to natural field conditions based on studies of potted plants in growth chambers. (BAZZAZ and MCCONNAUGHAY 1992). Also, longer term studies are needed to allow for possible acclimation responses.

REEKIE and BAZZAZ (1989) found that the two measures that explained almost 75% of the variation in competitive success were mean canopy height and leaf area ratio. Net leaf-level photosynthesis explained less than 9% of the variability in the data. Others (OBERBAUER *et al.* 1985, cited in ARNONE 1996) found that biomass accumulation was greater in plants grown under elevated CO_2 even though leaf-level photosynthesis was lower in these plants. All of these observations suggest that CO_2 induced alterations in plant morphology and plant development may be more useful predictors of species competitive success than changes in their photosynthetic performance (STEWART and POTVIN 1996). REEKIE and BAZZAZ (1989) found highly significant shifts in the contribution of individual tree species to community above ground biomass with increasing CO_2 concentration. These shifts had occurred even though CO_2 level had no effect on overall community above ground biomass or on leaf area index. They showed that the success of a species was positively related to its mean canopy height measured at harvest.

Therefore the aim of the present study was to determine the effect of elevated CO_2 on plant growth in two *Acacia* species. This was measured in terms of height increases over time, final plant height, plant mass, branch number and leaf area. These variables were expected to give some idea of the relative success of these two species under a future elevated CO_2 regime. The effects of rhizobial inoculation or absence thereof under elevated and ambient CO_2 were also determined (see Chapter 4).

2.2 Materials and Methods

Seeds of *Acacia nilotica* and *Acacia sieberana* were collected from plants growing in and around the suburbs of Bisley and Hayfields in Pietermaritzburg (located 735 metres above sea level, annual rainfall 700 - 1000 mm), KwaZulu-Natal, South Africa. Seeds were scarified and germinated on moist filter paper in petri dishes at room temperature. The seeds were then transferred to seedling trays filled with vermiculite and left to grow to a height of approximately 70mm and then transferred to the larger experimental pots. The plants were grown in acid washed sand, in 175 (height) x 150 (upper diameter) x 115mm (lower diameter) plastic pots, to allow accurate control of nutrient supply and *Rhizobium* flora which may have affected the experimental results adversely. Fifty plants of each species (one hundred per chamber, 200 altogether) were randomly assigned to two environmental growth chambers and maintained at approximately 370 $\mu l l^{-1}$ and 700 $\mu l l^{-1}$ CO_2, hereafter referred to as ambient (amb) and elevated (elev) CO_2. These CO_2 concentrations were used because they approximate present day and projected future CO_2 levels, and because they are commonly used in CO_2 studies (EAMUS and JARVIS 1989, COLEMAN and BAZZAZ 1992).

Within each CO_2 concentration treatment 25 plants of each species were inoculated with *Rhizobium* sp. and 25 plants were left uninoculated. The growth chambers were constructed out of steel frames hung with transparent plastic walls, and closed with the same plastic top. CO_2 was trickled into one chamber (the elevated CO_2 chamber) through a regulator at one end, along with compressed air, and was circulated within the chamber by a fan. The second (ambient) chamber received only compressed air, which was also circulated by a similar fan. The concentration of CO_2 within the chamber (specifically within the elevated CO_2 chamber, since it was determined that the levels of CO_2 in the ambient chamber were not affected by the experimental conditions and that the CO_2 concentration

remained at ambient levels throughout the experiment) was initially monitored daily to weekly, depending on its fluctuation, with an infra red gas analyser (LCA3, Analytical development Co. Ltd, Hoddesdon, England). At the same time, a "bubble-meter" was calibrated according to the required flow rate for the desired CO_2 concentration. Once the required CO_2 flow rate was established, (using the IRGA and bubble meter, the flow rate necessary to give the desired CO_2 concentration) the frequency of IRGA monitoring was decreased, and the flow rate controlled using the cylinder regulator and the bubble-meter. A bubble-meter is a modest piece of apparatus made from a modified pipet. The pipet is held upright by a retort stand. A small quantity of soapy water is retained in a small rubber balloon attached to the base of the pipet. CO_2 enriched air from the CO_2 cylinder, via the regulator, is intermittently fed into this bubble-meter from a point below some of the bubbles from the agitated soapy mixture. The time taken for a given bubble to move a certain distance gives an indication of the CO_2 flow rate. This flow rate was adjusted manually, daily, so as to give the requisite flow rate for the required concentration of CO_2 within the chamber. Weekly to bi-weekly measurements (using the IRGA) of the CO_2 concentration within the chambers indicated that while not accurate, the required CO_2 concentrations (within 15 - 20 %) were being maintained.

Plants were inoculated by watering them with an infusion of the soil from beneath either *A. nilotica* or *A. sieberana.* An infusion was prepared by soaking the soil overnight in 25 litres of water, which was then filtered through 2 layers of muslin cloth. The seedlings/saplings were watered to saturation twice a week and 50 ml 60% Hoagland's nutrient solution applied twice a week (**Table 2.1**). The nutrient solution was prepared by weighing the required amounts (**Table 2.1**) of each nutrient and dissolving these in one litre of distilled water. These solutions were maintained at -10°C until use. Prior to feeding the plants, 25 litre blackened water containers with taps were filled to the 10 litre mark with distilled water. One hundred millilitres of each stock solution was added to the water container, and mixed thoroughly, to give a 10% concentration of nutrient solution. After two months the nitrogen supply was decreased by supplying the plants with the original Hoagland's solution once every two weeks, and a modified Hoagland's solution (in which the nitrogen had been removed) for the rest of the study.

Due to the growing conditions within the chamber (hot and humid), the sand in which the plants were growing was always moist to some degree. The temperatures within the

chambers in mid-summer ranged between 30 and 40°C. Some of the plants were scorched by this heat, and lost a small amount of leaf material. Plants were rotated within the chambers every two weeks, so as to nullify edge effects within the chamber. The saplings were grown in this way over a period of seven months during which time growth rates (change in height and height at harvest) and photosynthetic rates were monitored. The same plants were used throughout the study, for all experiments except for the Drakensberg transplant experiment (Chapter 6).

Table 2.1. Composition of Hoagland's nutrient solution according to HOAGLAND and SNYDER (1933). Mixed to give a 10 % final concentration (100ml / 10 litres).

Stock Solution	Concentration	Mass l^{-1}
$Ca(NO_3)_2.4H_20$	0.75M	177g
KNO_3	0.75M	76g
$MgSO_4.7H_2O$	0.30M	74g
KH_2PO_4	0.15M	20.4g
NaFeEDTA	2.3 mM	840mg
Micronutrients		
H_3BO_3	7.0mM	430mg
$MnCl_2.4H_2O$	1.37mM	272mg
$ZnSO_4.7H_20$	0.12mM	33mg
$CuSO_4.5H_2O$	22 µM	12mg
$H_2MoO_4.H_2O$	16 µM	3mg
$NaNO_3$	0.75M	81g
$MgCl_2$	0.30M	28.6g
$NaSO_4$	0.30M	42.6g
NaH_2PO_4	0.15M	18.0g
$CaCl_2$	0.75M	83.2g
KCl	0.75M	55.9g

At the end of the experiment, leaf, stem and root masses were determined. The plants were initially weighed upon harvesting and then dried at 75-80°C for 48 hours, or until equilibrium was reached, and then re-weighed. Total plant leaf area was determined using a LI-3000 leaf area meter (Li-COR Inc. Lincoln, Nebraska). Unfortunately it was not possible to move the leaf area meter into the greenhouse, and this resulted in (relatively) lengthy waiting times between harvesting and measurement. This resulted in the closing of some of the leaves, which reduced leaf area measurements in some instances. This could not be resolved by carrying the plants upstairs, as the shock of movement, and exposure to interior conditions had the same effect on the plants as on cut leaves. The

best solution was to speed up time between harvesting and measurement. The leaves were placed in plastic bags between harvesting and measurement to reduce water loss to transpiration.

Root nodule formation was determined by visual inspection. All the plants that were initially inoculated were observed to be nodulated at the final harvest. When the 'uninoculated' plants became 'infected' with rhizobial root bacteria towards the end of the experiment, the experiment was terminated.

2.3 Results

2.3.1 Plant Mass

After seven months, seedlings/saplings (particularly *Acacia nilotica*) grown at elevated CO_2 were larger than those grown at ambient CO_2. Total plant mass increased in response to CO_2 enrichment by 33%* ($P \leq 0.005$) and 40% ($P \leq 0.001$) in seedlings of *Acacia sieberana* and *Acacia nilotica* respectively (**Tables 2.2 and 2.3**). The elevated CO_2 treatment significantly increased the separate stem, leaf and root dry and fresh masses ($P \leq 0.001$ for all) in *A. nilotica*. Stem ($P < 0.01$), leaf ($P = 0.03$) and root ($P < 0.01$) dry masses were also increased in *A. sieberana*. *A. sieberana* root ($P < 0.001$) and stem ($P = 0.01$) fresh masses were significantly increased (**Table 2.2**), Leaf fresh mass was also increased, although not significantly.

The effect of root inoculation with rhizobial root nodule bacteria was the most influential factor across all treatments with CO_2 enrichment increasing this positive effect. The results for most measurements; total mass, separate stem, leaf and root fresh and dry mass, leaf area and plant height were all highly significant ($P < 0.001$) across all treatments, in general, elevated CO_2 levels and inoculation with *Rhizobium* sp. increased plant mass.

* Percentage increase = increased value - ambient or lower value = x

= x / ambient or lower value.(100)

2.3.2 Leaf area

Some of the treatment combinations exhibited significant differences in leaf area measurements (**Tables 2.2 and 2.3**, Multifactor ANOVA, $P \leq 0.001$). Leaf area of *Acacia*

nilotica, grown in elevated CO_2, increased by 26% ($P < 0.005$) compared to plants grown under ambient CO_2 conditions, while inoculation increased leaf area by 138% ($P < 0.0001$) compared to uninoculated plants. Similar results were obtained for *Acacia sieberana*, with smaller differences between ambient and elevated CO_2 plants (24% increase in elevated CO_2, $P < 0.1$, n.s., when including both inoculation treatments) and inoculated and uninoculated plants (48% increase in leaf area of inoculated plants, $P < 0.005$). Within the treatments however, the presence of root nodules seemed to have the greatest positive effect on leaf area, with elevated CO_2 enhancing this effect on leaf area. Thus, in most instances the greatest leaf areas were found firstly in inoculated plants, and secondly in elevated CO_2 plants. In *A. sieberana*, however, inoculated plants from the ambient chamber had greater leaf areas than inoculated plants from the elevated CO_2 chamber. Uninoculated plants in both species had significantly greater leaf areas when exposed to elevated CO_2 (**Table 2.3**).

Table 2.2. The effect of CO_2 concentration (ambient or elevated) and rhizobial inoculation on the allocation of mass to various plant parts of *Acacia sieberana* and *Acacia nilotica*. A two-way analysis of variance was carried out for each species (* $P < 0.05$, ** $P < 0.01$, n.s. not significant).

Treatment	Factor	Dry mass	Fresh mass	Leaf area
A. sieberana				
Leaf	CO_2 level	*	n.s.	n.s.
	Inoculation	**	**	**
	CO_2 x inoculation	n.s.	n.s.	n.s.
Stem	CO_2 level	**	*	-
	Inoculation	**	**	-
	CO_2 x inoculation	n.s.	n.s.	-
Root	CO_2 level	**	**	-
	Inoculation	**	**	-
	CO_2 x inoculation	n.s.	n.s.	-
A. nilotica				
Leaf	CO_2 level	**	**	**
	Inoculation	**	**	**
	CO_2 x inoculation	n.s.	n.s.	n.s.
Stem	CO_2 level	**	**	-
	Inoculation	**	**	-
	CO_2 x inoculation	*	**	-
Root	CO_2 level	**	**	-
	Inoculation	**	**	-
	CO_2 x inoculation	n.s	n.s.	-

2.3.3 *Plant height*

Final plant heights in both species were greater in plants grown in elevated CO_2 and with rhizobia. This was most significant in *A. nilotica*. However, the presence of rhizobia was again the most powerful determinant. That is, in both species, plants grown with rhizobia were on average 36% taller than those without, irrespective of the CO_2 concentration. Plants grown in elevated CO_2 were taller (21% for *A. sieberana* and 14% in *A. nilotica*) than their presence/absence rhizobia counterparts grown in ambient CO_2 (**Table 2.3**). This was a trend established early on in the experiment. Photographic evidence of the typical responses in each group was gathered and is presented in **Figures 2.1 - 2.6**. The rhizobia effect diminished as the experiment progressed, possibly due to cross-contamination, at which point the experiment was terminated.

Table 2.3. The total plant mass (dry), root mass (dry), average height (at harvest), average number of branches per plant, average leaf area, and root:shoot ratio in *Acacia sieberana* and *Acacia nilotica* grown at elevated (elev) or ambient (amb) levels of CO_2, and inoculated (+) or uninoculated (-) with root nodule bacteria. Analysis of variance was carried out for both species, for each species, within each column, means followed by different letters are significantly different ($P < 0.05$).

Treatment	Total plant mass (g)	Root mass (g)	Leaf area (cm^2)	Height (mm)	Branch number	Root: Shoot ratio
A. sieberana						
elev, +	19.31 ^{c}	10.56 ^{c}	485.71 ^{b}	841 ^{c}	0.25 ^{a}	1.60 ^{a}
elev, -	12.80 ^{a,b}	7.44 ^{a,b}	416.65 ^{a,b}	638 ^{a,b}	0.60 ^{a}	1.90 ^{a,b}
amb, +	15.02 ^{b,c}	8.18 ^{b,c}	488.04 ^{b}	714 ^{b,c}	0.20 ^{a}	1.63 ^{a,b}
amb, -	9.18 ^{a}	5.91 ^{a}	239.00 ^{a}	507 ^{a}	0.36 ^{a}	2.22 ^{b}
A. nilotica						
elev, +	17.98 ^{c}	7.04 ^{c}	373.59 ^{c}	711 ^{c}	10.25 ^{c}	0.64 ^{a}
elev, -	8.38 ^{a}	3.49 ^{a,b}	191.80 ^{b}	512 ^{a}	6.88 ^{b}	0.75 ^{b,c}
amb, +	12.65 ^{b}	5.32 ^{b,c}	339.46 ^{c}	613 ^{b}	6.80 ^{b}	0.73 ^{b}
amb, -	6.27 ^{a}	2.89 ^{a}	107.22 ^{a}	456 ^{a}	2.50 ^{a}	0.91 ^{c}

2.3.4 *Branch number*

Branch numbers were only increased significantly in *A. nilotica* ($P \leq 0.001$). Plants grown in elevated CO_2 had 84% more branches than those grown in ambient conditions, while the presence of rhizobia enhanced this effect by 82% (**Table 2.3**). *A. sieberana* had very few branches, in all treatment combinations.

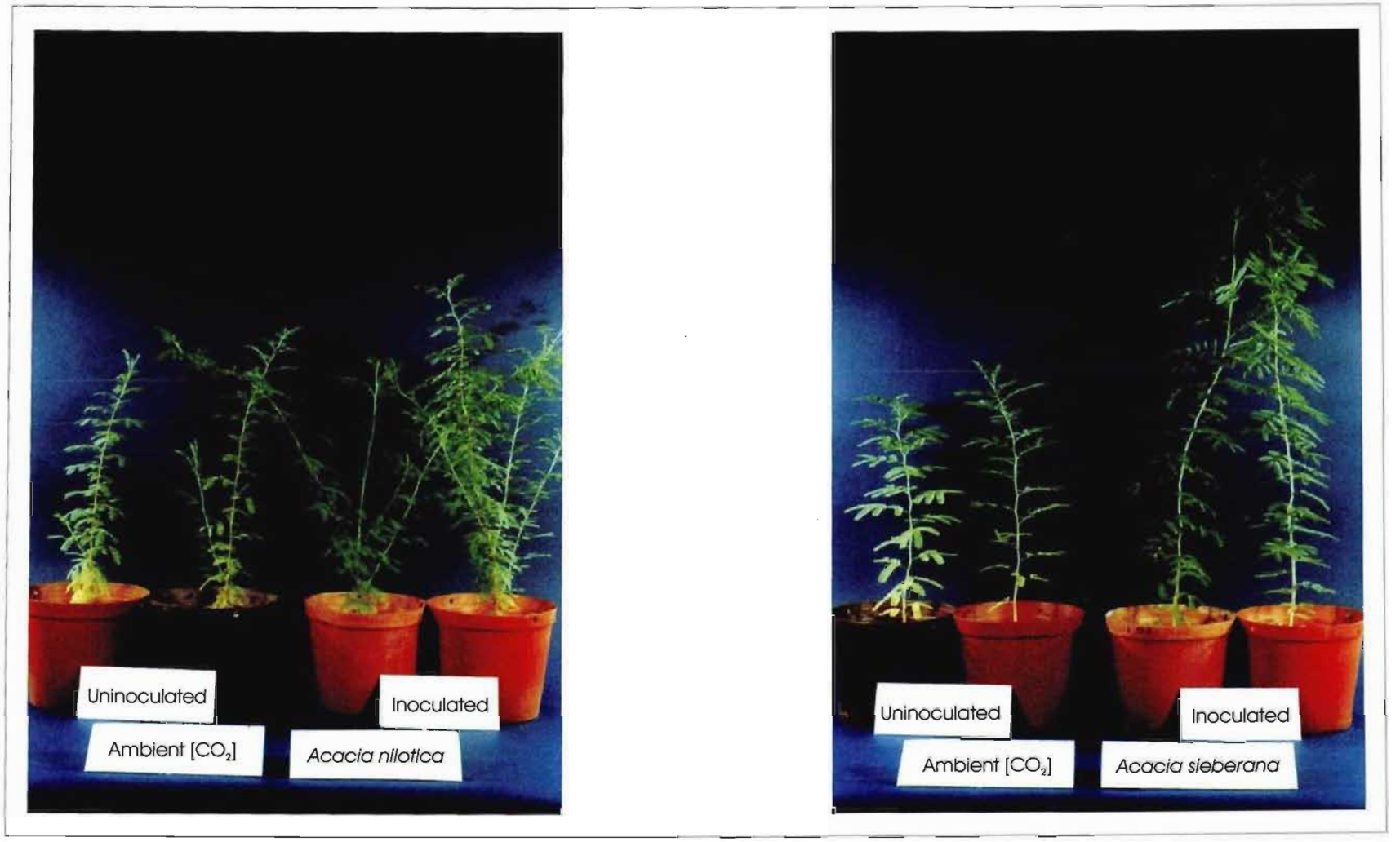

Figure 2.1. Visual differences between *Acacia nilotica* and *Acacia sieberana* grown under ambient conditions, either inoculated or uninoculated with *rhizobium* root nodule bacteria. Note the increases in branching and height in *Acacia nilotica* and *Acacia sieberana* respectively.

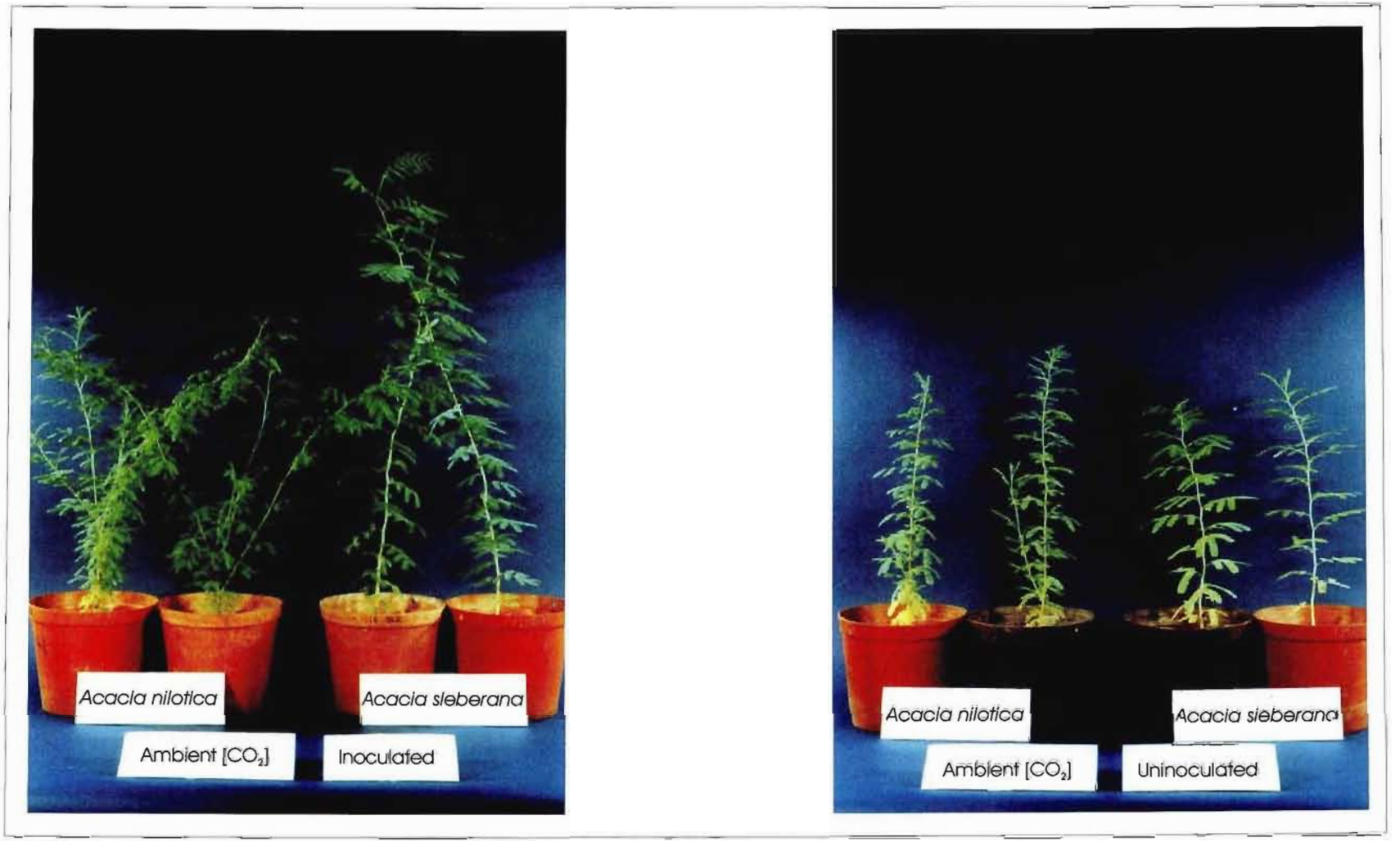

Figure 2.2. The differences between plants grown under ambient CO_2 conditions when inoculated and uninoculated in both *Acacia nilotica* and *Acacia sieberana*.

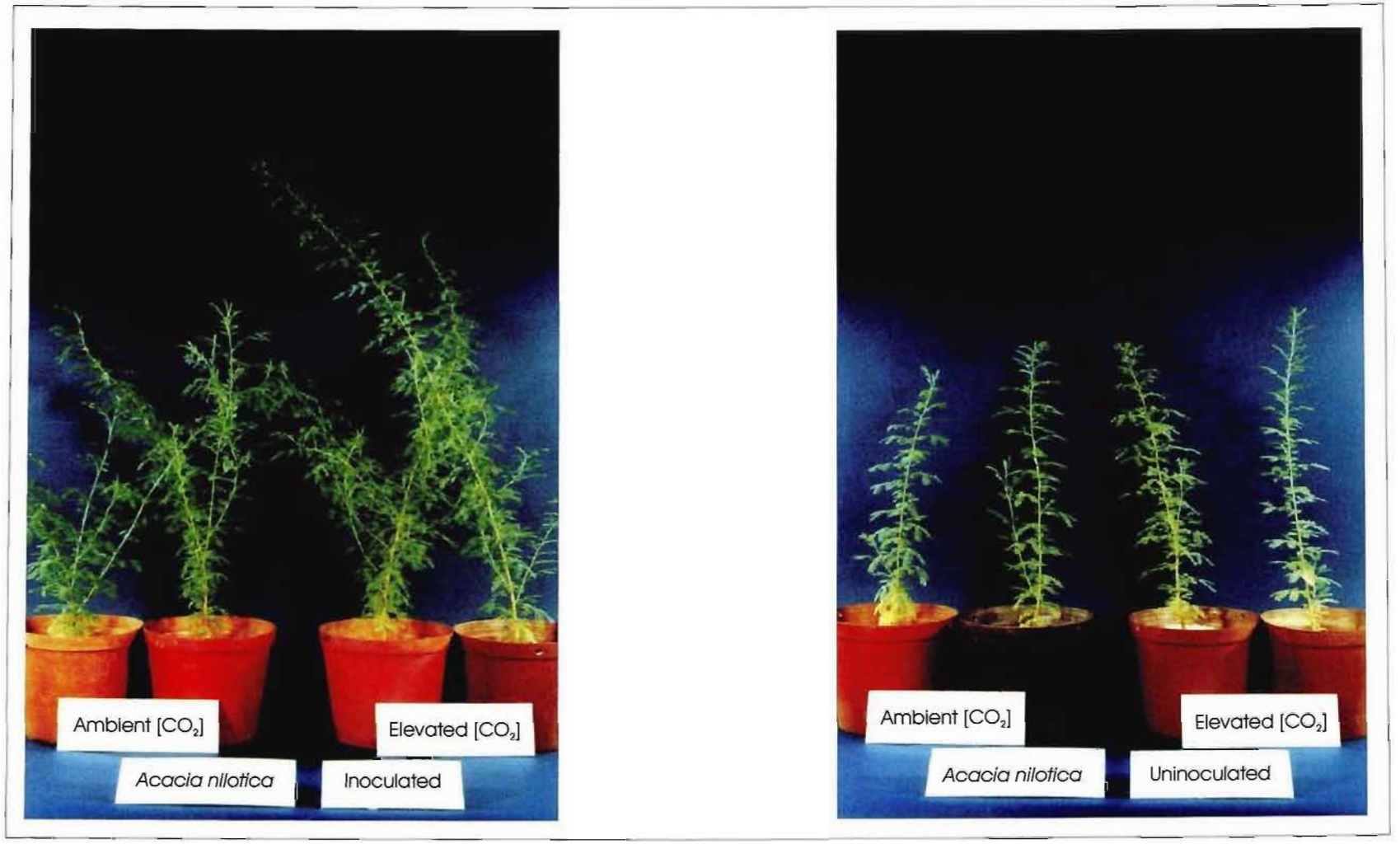

Figure 2.3. The differences between *Acacia nilotica* inoculated and uninoculated plants grown under both ambient and elevated CO_2 conditions. Notice how the differences between elevated and ambient CO_2 grown plants are more pronounced under inoculated conditions.

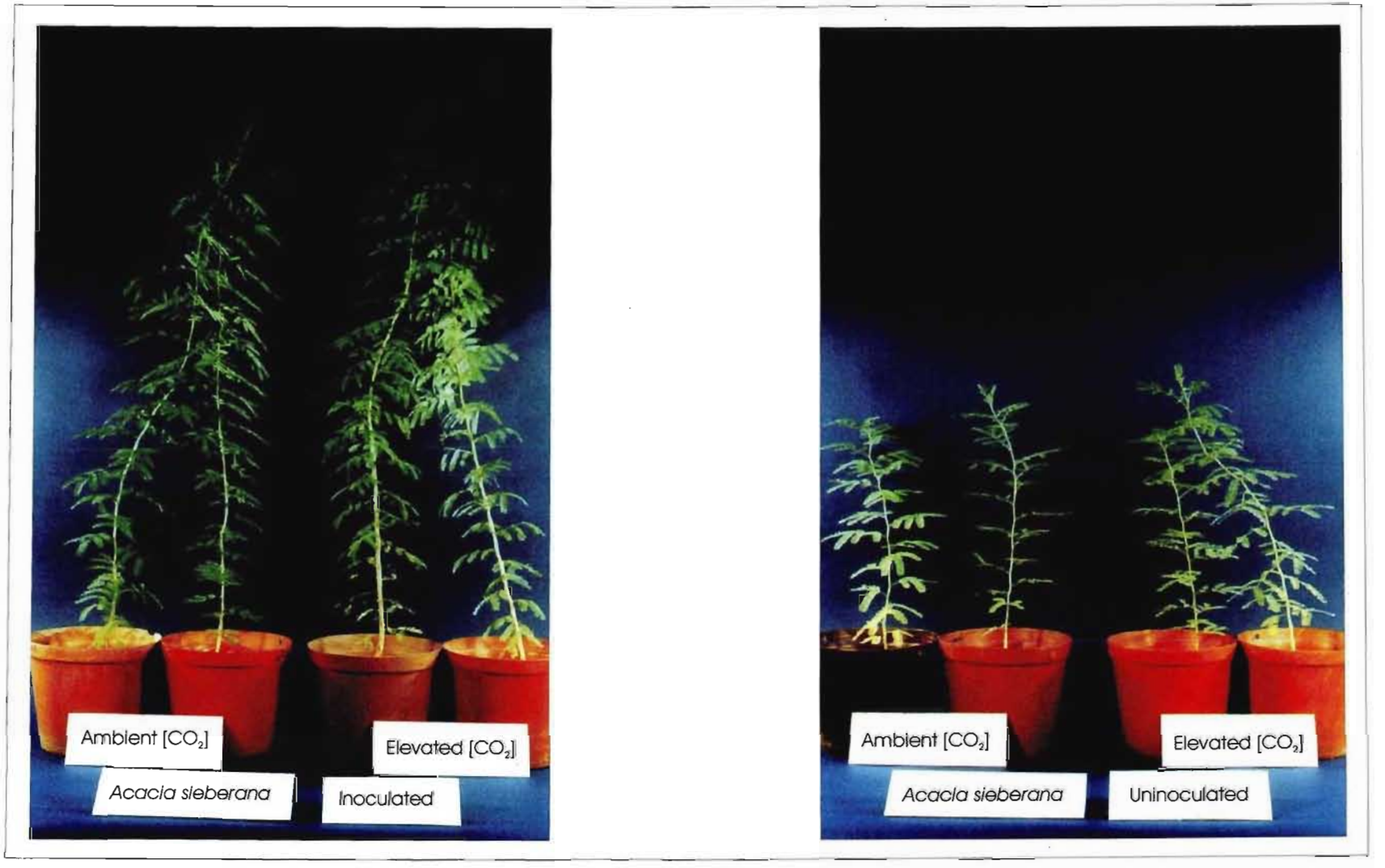

Figure 2.4. The differences between *Acacia sieberana* inoculated and uninoculated plants grown under elevated and ambient CO_2 conditions. Notice how the differences between inoculated and uninoculated plants are more obvious than the differences between elevated and ambient CO_2 treatments.

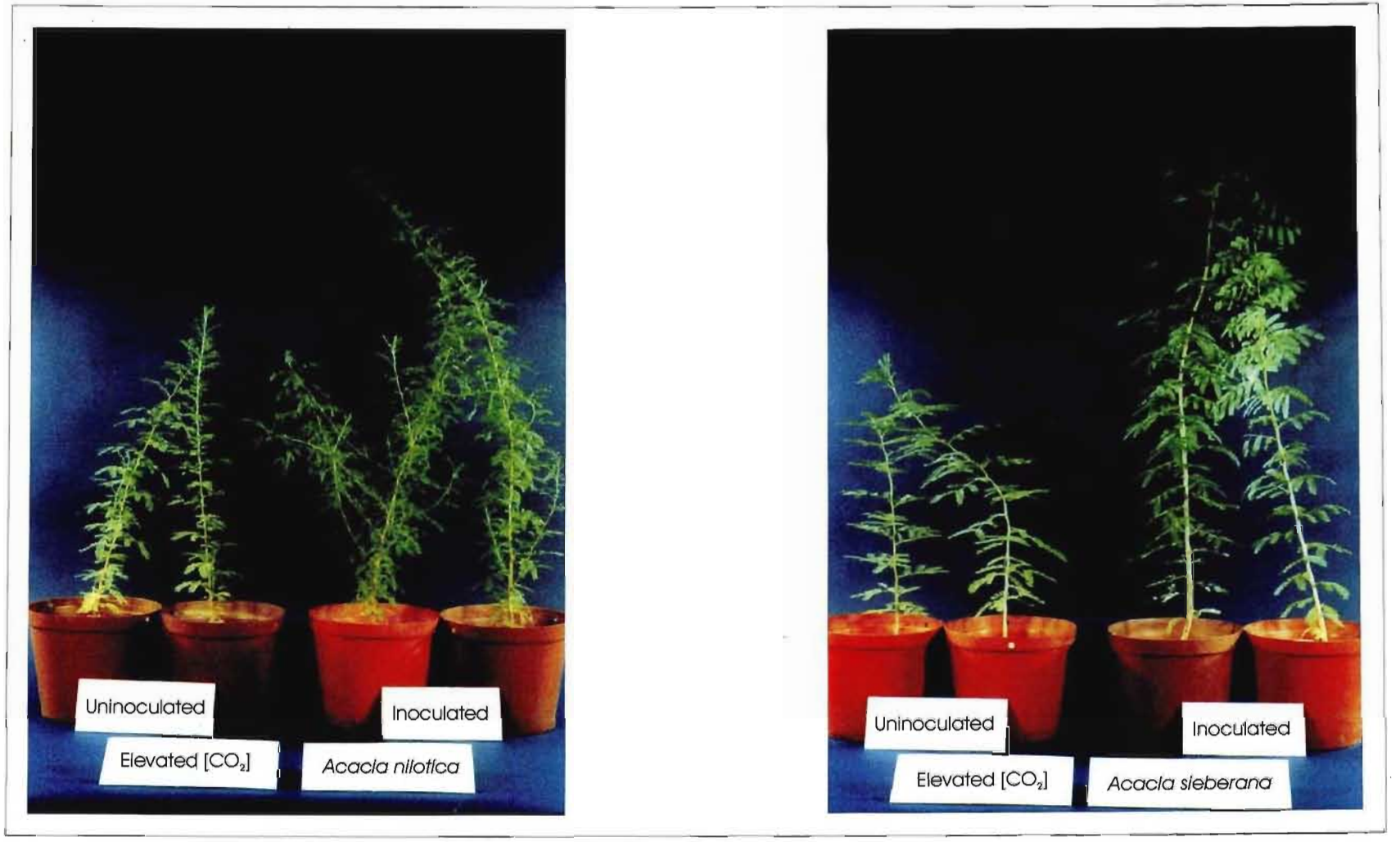

Figure 2.5. Visual differences between *Acacia nilotica* and *Acacia sieberana* grown under elevated CO_2 conditions, either inoculated or uninoculated with *rhizobium* sp. Note the effects of rhizobial inoculation on branching and height in *Acacia nilotica* and *Acacia sieberana* respectively.

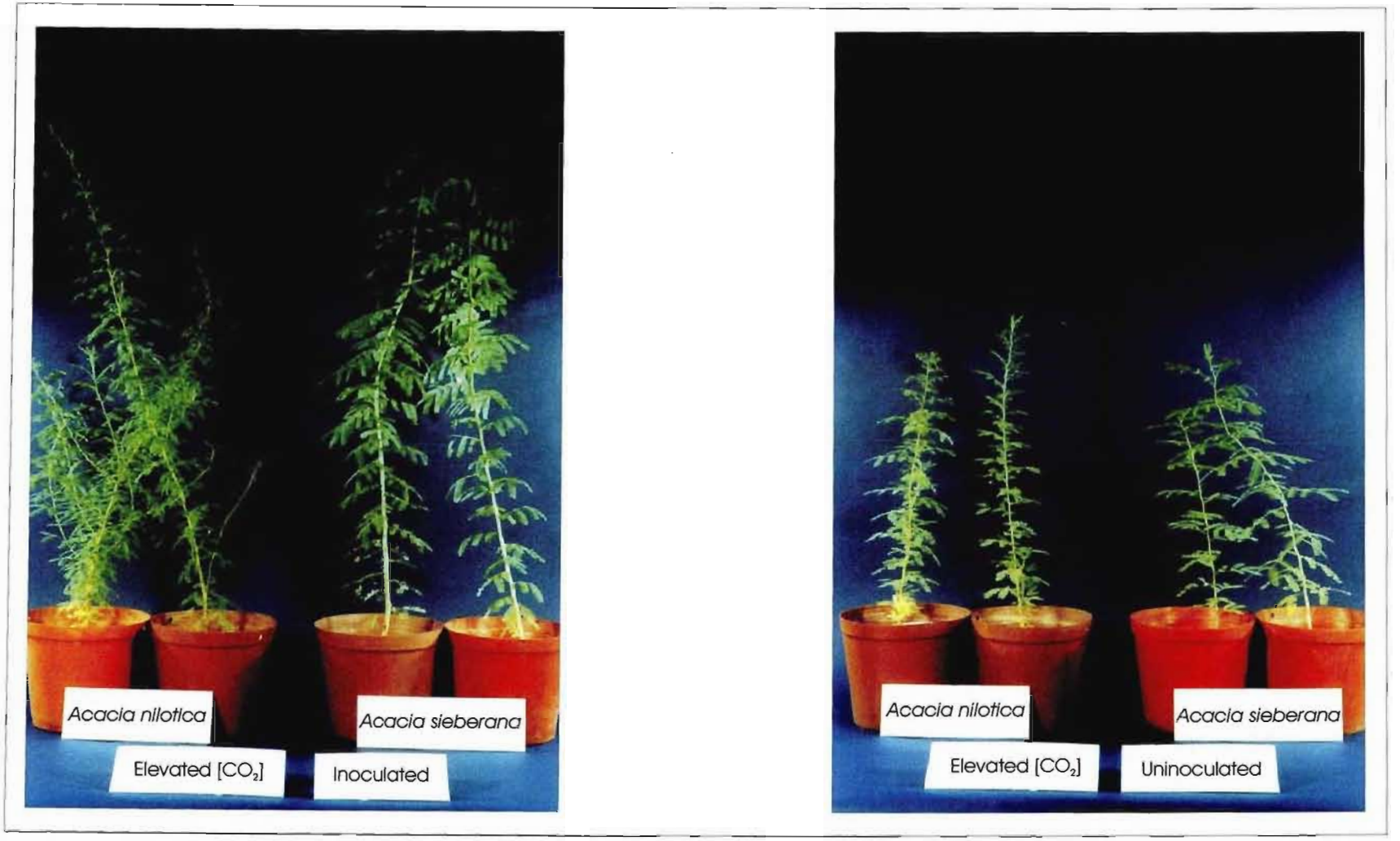

Figure 2.6. Visual differences between plants grown under elevated CO_2 conditions when inoculated and uninoculated in both *Acacia nilotica* and *Acacia sieberana*.

2.3.5 *Root:shoot ratios*

Root:shoot ratios were greatest in plants grown without rhizobial inoculation in both CO_2 treatments, and were, on average, higher in ambient CO_2 treatments than elevated CO_2 treatments for both species (**Table 2.3**).

2.4 Discussion

The results presented in this study show that a doubling of CO_2 concentration can increase *Acacia* growth (**Tables 2.2 and 2.3, Figures 2.3 and 2.4, and Figures 2.2 and 2.6**) and thus mass accumulation, but these increases were most significant when the Acacias were inoculated with rhizobia. Growth increased despite minimal increases in photosynthetic rates in *A. nilotica* and reduced photosynthetic rates in *A. sieberana* under elevated CO_2 as compared to ambient CO_2. Possible reasons for this apparently anomalous result are discussed in Chapter 3.

Total plant mass increased by an average of 36.5% across the two species, with *A. nilotica* increasing more than *A. sieberana* (**Table 2.3**). Many of the beneficial effects of CO_2 enrichment on plants resemble those following the application of fertiliser (JONES *et al.* 1996). Principally, the elevated levels of CO_2 stimulate photosynthetic fixation, which in turn stimulates growth and respiration as well as further enhancing carbohydrate supply to the root nodule bacteria. Traits that may affect competitive ability in an elevated CO_2 environment are photosynthetic pathway, mycorrhizae and N_2 fixation (REYNOLDS 1996). In this study root inoculation had the greatest affect on plant mass accumulation across all treatments with CO_2 increasing this effect.

The growth of rhizobial-inoculated plants responded positively to the enrichment of the rhizosphere with CO_2. Inoculated plants usually showed higher rates of nitrogen and CO_2 uptake than equivalent plants grown in ambient CO_2 (CRAMER *et al.* 1996). The results obtained in this study indicate that CO_2 had little effect on the percentage leaf nitrogen but that rhizobial inoculation (or N availability) was again the dominant factor (this will be discussed in more detail in Chapter 4). There is little doubt that elevated CO_2 will increase primary production, but due to the already high concentrations of CO_2 present in soil, it is unlikely that a doubling of atmospheric CO_2 will directly affect soil processes (HENNING *et*

al. 1996). It is possible that CO_2 enrichment will alter the quality of organic matter and thereby alter the rates at which carbon and nitrogen are cycled in plant systems (BAZZAZ 1990, HENNING *et al.* 1996).

REEKIE and BAZZAZ (1989), ARNONE (1996) and WAND *et al.* (1996) concluded that the success of a species was positively related to its mean canopy height and that leaf and canopy morphological changes could alter patterns of resource availability. Plant height, and mass of the plants in this study increased in both species when they were exposed to elevated CO_2 and rhizobial inoculation, this was a trend-like increase, and was only highly significant in *A. nilotica*, and in inoculated plants. The increase in leaf area caused by elevated CO_2 was not significant in *A. sieberana* (**Table 2.2**) and decreased in inoculated plants. Rhizobial inoculation contributed significantly to growth and subsequent increases in mass and height. VOLIN and REICH (1996) found that photosynthetic rates were significantly greater for plants grown with high nitrogen compared to those grown in low nitrogen. MITCHELL *et al.* (1995) found that increased nitrogen and CO_2 supplies and the interaction of these two factors markedly increased respiration rate. However, nitrogen supply had a greater effect on both respiration and growth than did atmospheric CO_2 concentration (GRIFFEN *et al.*1993, MITCHELL *et al.* 1995) .

Differential shoot morphological response to increased CO_2 (and nitrogen if inoculated) such as changes in leaf area, branching and tillering may lead to changes in canopy architecture. This may alter the species competitive balance by modifying foliar light interception patterns (BAZZAZ and MCCONNAUGHY 1992; LUO and MOONEY 1995) which may be particularly important during seedling establishment (WAND *et al.* 1996). Root:shoot ratio (RSR) is commonly used to assess compensatory changes in root growth characteristics in response to CO_2 enrichment. RSRs are often (DAY *et al.* 1996) but not always increased under elevated CO_2. This ratio is an important index of compensatory changes in carbon allocation (and a useful overall measure of treatment response) and a poor index of plant potential for nutrient acquisition (POORTER 1993; BASSIRIRAD *et al.* 1996). In this study RSRs were greatest in plants grown without rhizobial inoculation in both CO_2 treatments (**Table 2.3**). RSRs were also higher under ambient CO_2 when compared to elevated CO_2 treatments in both species. Allocation of photoassimilates between shoots and roots is partly determined by genetics, but also changes adaptively, with greater allocation to roots under nutrient and water stress (NORBY *et al.* 1986; KORNER

and ARNONE 1992; HUNT *et al.* 1996). The absence of rhizobial inoculation and CO_2 enrichment seemed to have a similar 'low nutrient' effect on the growth of both species. This effect was comparatively equally pronounced in both species, suggesting that both species will fare equally well under conditions of nutrient or water stress, regardless of the CO_2 concentration, if their root systems can grow extensively, and if plants are inoculated with root nodule bacteria. If temperature and water status are unchanged, an increase in CO_2 might increase RSR by way of the plant's adaptive response to decreasing carbon limitation relative to nutrient limitation. However, elevated CO_2 may also improve plant water status thereby affecting RSR (HUNT *et al.* 1996). Rhizobial inoculation will become especially important in determining a plant's success under nutrient stress if the RSR is affected significantly.

In *A. nilotica* elevated CO_2 and inoculation increased branching. Enhanced numbers of specific parts (stems, branches, tillers and flowers) have often been reported in response to elevated CO_2 concentration (ROGERS and DAHLMAN 1993). The small differences between *A. sieberana* treatments with respect to branching confound the results. Although there were more branches on plants grown without rhizobial nodules, the reason for this is unclear. Other studies have found that some plants grown under elevated CO_2 conditions are not able to convert additional photosynthate into increased growth. For these plants no changes in total biomass, accumulations of non-structural carbohydrates in leaves, leaf discolouration, and increased below ground carbon occurred (DIAZ *et al.* 1993, cited in REYNOLDS 1996). These studies were however, usually conducted under low fertility conditions. Species with nitrogen-fixing ability or mycorrhizae may be able to avoid competition for soil nutrients, and demonstrate potential growth responses to elevated CO_2 (REYNOLDS 1996). In the present study this was demonstrated where inoculated plants showed greater increases in mass, and height than uninoculated plants.

REEKIE and BAZZAZ (1989) found highly significant shifts in the contributions of individual tree species to community above ground biomass with increasing CO_2 concentration. These shifts occurred even though the CO_2 level had no effect on above ground biomass or on leaf area index. Thus, it may be assumed that even greater changes in the allocation of carbon to various plant parts will occur in plants such as Acacias which show obvious changes under experimental conditions. These increases in carbon allocation will inevitably lead to changes in light interception, nutrient acquisition, species composition

and plant distribution. Since CO_2 affects a wide range of plant functions both directly and indirectly, predicting the direction, much less the magnitude, of changes in plant function and community structure is difficult. This is because there is a lack of understanding of the mechanisms that control overall plant response to CO_2 concentration (MITCHELL *et al.* 1995).

Shifts may also occur in species composition in favour of nitrogen-fixing species. This suggests that when species are differentially infected, nitrogen-fixing ability will be important in understanding shifts in species composition in response to elevated CO_2, even when soil fertility is too low to support a community level response (REYNOLDS 1996). As a demonstration of this phenomenon, POORTER (1993) reported for data compiled from 106 species, an average CO_2 induced mass increase of 41%, which was increased to 50% in the case of nitrogen-fixing species. Evidently nitrogen fixing species will be favoured in a CO_2 enriched world, and the results obtained in this study confirm this supposition.

2.5 Conclusions

Because CO_2 enrichment generally results in increased photosynthetic fixation, the possibility exists for increased carbon storage in an elevated CO_2 world. Increased plant biomass in some species would ultimately result in decreased biomass in others, altering the competitive balance and changing species composition in both plant and animal species. This would be influenced by factors such as temperature, light, rhizobial status of the soil and plants, water availability, photosynthetic pathway, and all other typically encountered limiting factors. Plants which can withstand long periods of drought, fix nitrogen, or use CO_2 more efficiently are likely to emerge as the victors in any interspecific competition.

This study has revealed that while CO_2 fertilization has significant effects on plant mass height and leaf area accumulation, other factors, such as species type and rhizobial inoculation have equally, if not greater influence on the short term (longer-term experiments (e.g. lasting years) may not show the same effect) mass accumulation under elevated CO_2. Thus the presence of rhizobia in the soil and the ability of plants to use this advantageously will have increased importance in an elevated CO_2 environment.

CHAPTER 3

Photosynthetic response curves and stomatal conductance

3.1 Introduction

3.1.1 *Photosynthetic response curves*

The comparative analysis of CO_2 and water vapour exchange between the atmosphere and leaves of plants growing at varying concentrations of atmospheric CO_2 is an important procedure for assessing photosynthetic acclimation to elevated CO_2. A/C_i analyses facilitate the study of acclimation because they are conducted at equivalent measurement conditions and can be interpreted in terms of the biochemical and stomatal processes controlling the long-term response of photosynthetic capacity to CO_2 enrichment (SAGE 1994). However, photosynthesis may not be enhanced at all, despite increases in relative biomass. Changes in community structure may be affected despite little or no change in productivity in most species, simply as a result of increased productivity in some species (BAZZAZ and MCCONNAUGHAY 1992). Thus the measure of photosynthetic rate is relative, dependent on species, and largely unreliable as a gross measure of potential change. Leaf gas exchange analysis non-destructively describes primary and secondary responses of photosynthesis to CO_2 enrichment and can be used to assess the biochemical and stomatal mechanisms controlling short and long term responses of leaves to elevated CO_2 (SAGE 1994).

3.1.2 Stomatal Conductance

A frequent response of plants to elevated CO_2 is partial stomatal closure, which may have important implications for canopy temperature, water use and growth under elevated CO_2 and water limited conditions (POORTER 1993; ELLSWORTH *et al.* 1995; WILSEY 1996). However, many tree species exhibit relatively insensitive stomatal responses to CO_2 with long term exposure (BUNCE (1992) cited in ELLSWORTH *et al.* 1995). While the hypothesis that stomatal conductance is regulated in proportion to the photosynthetic demand for CO_2 within the leaf is supported by much experimental evidence from studies of herbaceous plants, there is little data from trees to test the hypothesis (ELLSWORTH *et al.* 1995).

3.2 Materials and Methods

The plants (and associated Materials and Methods) described in Chapter 2 were also used in this experiment. The plants were approximately five months old when this particular experiment commenced. A LCA3 portable infrared CO_2 gas analyser (IRGA) (LCA3, Analytical development Co. Ltd, Hoddesdon, England) was used to measure net assimilation rates (NAR). NAR was measured on expanded leaves of the same age and position on 6 different plants from each CO_2 and rhizobial treatment (24 replicates in total). These leaves were enclosed in an artificially illuminated (at a previously established optimum irradiance (see last paragraph of Materials and Methods, below) for photosynthesis of 600-700 μmol $m^{-2}s^{-1}$) temperature controlled and ventilated cuvette. Foliage was pre-illuminated for 20-30 minutes prior to beginning measurements to ensure that the stomatal opening response to light was complete.

Measurements were made at leaf temperatures comprised between 18.5 and 21°C. The humidity within the cuvette was kept at a constant 50 ± 2%. Measurements were begun when the CO_2 concentration stabilised at pre-selected levels and A_{net} had equilibrated at each new CO_2 level for 5-10 minutes. $A:C_i$ response was measured by changing the CO_2 concentration of air entering the cuvette (C_a) into 13 steps (50, 100, 150, 200, 250, 300, 350, 400, 500, 600, 700, 800, 900μll^{-1}) and recording gas exchange parameters when all variables were steady. Three sequential A_{net} measurements were recorded for each of the thirteen CO_2 concentrations listed above. In addition, several variables were automatically recorded by the IRGA with each manual recording of A_{net}. Thus each other variable was also recorded three times per measurement. These measurements were stored in the IRGA, and were downloaded and saved to disc at the same time as the photosynthetic rate readings. In the A/C_i curves presented in **Figures 3.1 and 3.2** the points represent fitted values, and the error bars the 95% confidence limits calculated using the "Spline" program of HUNT and PARSONS (1974). The variables measured included: set number, plot, record, day, hour, relative humidity (RH) In, RH out, reference [CO_2], analysis [CO_2], photosynthetically active radiation (PAR), air temperature, transpiration (E), leaf temperature, stomatal conductance, photosynthetic rate (A), leaf internal [CO_2] C_i and flowrate. From these variables corrected A, mean PAR and mean A were determined for

each treatment. The leaf areas of the portions of leaf enclosed in the cuvette were determined using a LI-3000 leaf area meter (Li-COR inc. Lincoln, Nebraska).

In addition to A:C_i curves, a set of 16 measurements (four for each species and CO_2 combination) were taken, using constant C_a values of 340 $\mu l l^{-1}$ (approximately ambient in the laboratory) and a range (80, 150, 250, 350, 450, 550, 650, 750, 850, 950 $\mu mol\ m^{-2}s^{-1}$) of light intensities. This was done to determine the optimum light intensity for photosynthesis.

3.3 Results

3.3.1 Photosynthetic response curves (A/C_i)

There were no significant differences in photosynthetic rates between ambient and elevated CO_2 grown plants of both *A. nilotica* and *A. sieberana* when compared across all CO_2 levels (**Figure 3.1**) although the variability in the results could have contributed to the smoothing out of differences. When photosynthesis was plotted against internal CO_2 concentration (A/C_i), the initial slopes of the graphs for both *A. sieberana* and *A. nilotica* were less for plants grown in elevated CO_2 compared to plants grown in ambient conditions. Plants of *A. nilotica* had greater photosynthetic rates when grown in elevated CO_2 as compared to ambient CO_2. Uninoculated *A. nilotica* grown in elevated CO_2 had anomalous results throughout the photosynthetic rate experiments. These plants may have been affected differently by growth under elevated CO_2 as compared to the other species and treatments. All the plants also reacted very strongly to any movement: from the greenhouse to the laboratory, and movement and changes in light intensity within the laboratory. *Acacia* leaves close quickly and easily with any change in stimulus. It may have been that sufficient time was not allowed between leaf responses and 'equilibration' back to 'normal' in some instances, resulting in anomalous results. However, all effort was made to ensure that equilibration was the same in all specimens, this was determined visually. Leaves were deemed to be 'equilibrated' once fully opened. Inoculated plants (both ambient and elevated CO_2) had higher photosynthetic rates than uninoculated plants (**Figure 3.1**) in both species (except for the uninoculated elevated CO_2 *A. nilotica* which had the highest photosynthetic rate of all). In *A. sieberana* the pattern was reversed. However when compared at the internal CO_2 level at which they were grown (C_a), a more

interesting trend emerged. C_i /C_a levels were plotted against each other, the ambient growth CO_2 level (C_a) at which the plants were grown (360 (amb) /700 $\mu l l^{-1}$(elev)) was extrapolated to this plotted line to find the corresponding internal CO_2 level (C_i) for that C_a. See arrows 1 & 2, **Figure 3.1**. When photosynthetic rates were compared at the levels of external CO_2 at which the plants were grown, plants grown in ambient CO_2 photosynthesised at a slightly higher rate than plants grown at the higher CO_2 concentration.

3.3.2 Stomatal conductance

Analysis of the variability (ANOVA) in stomatal conductance revealed only one obvious significant difference between the two species (**Table 3.1**). *A. nilotica* had greatly reduced stomatal conductance (mean stomatal conductance = 25.5 mol m^{-2} s^{-1}) when compared to *A. sieberana* (mean stomatal conductance = 57.3 mol m^{-2} s^{-1}). Within *A. nilotica* treatments there were no significant differences between treatments. Only the results of the uninoculated plants grown in elevated CO_2 seemed to deviate from the other treatments. This may have been an artefact of the experimental procedure used to establish stomatal conductance as this treatment had very variable results.

Table 3.1. The effect CO_2 level (elev/amb) and rhizobial inoculation (present, absent) on stomatal conductance's (as derived from the photosynthetic rate experiment) of *A. sieberana* and *A. nilotica*. Figures followed by the same letter are not significantly different ($P < 0.05$). Analysis of variance was carried out for each species, n = 6 for each species and treatment. Stomatal conductances were averaged across all CO_2 concentrations measured.

Treatment	Stomatal conductance (mol m^{-2} s^{-1})
A. sieberana	
Elev. CO_2, with *Rhizobium*	66 ± 23 ^{d}
Elev. CO_2, no *Rhizobium*	41 ± 16 ^{c}
Amb. CO_2, with *Rhizobium*	80 ± 26 ^{e}
Amb. CO_2, no *Rhizobium*	40 ± 19 ^{c}
A. nilotica	
Elev. CO_2, with *Rhizobium*	35 ± 14 ^{bc}
Elev. CO_2, no *Rhizobium*	7 ± 15 ^{a}
Amb. CO_2, with *Rhizobium*	30 ± 16 ^{b}
Amb. CO_2, no *Rhizobium*	31 ± 12 ^{b}

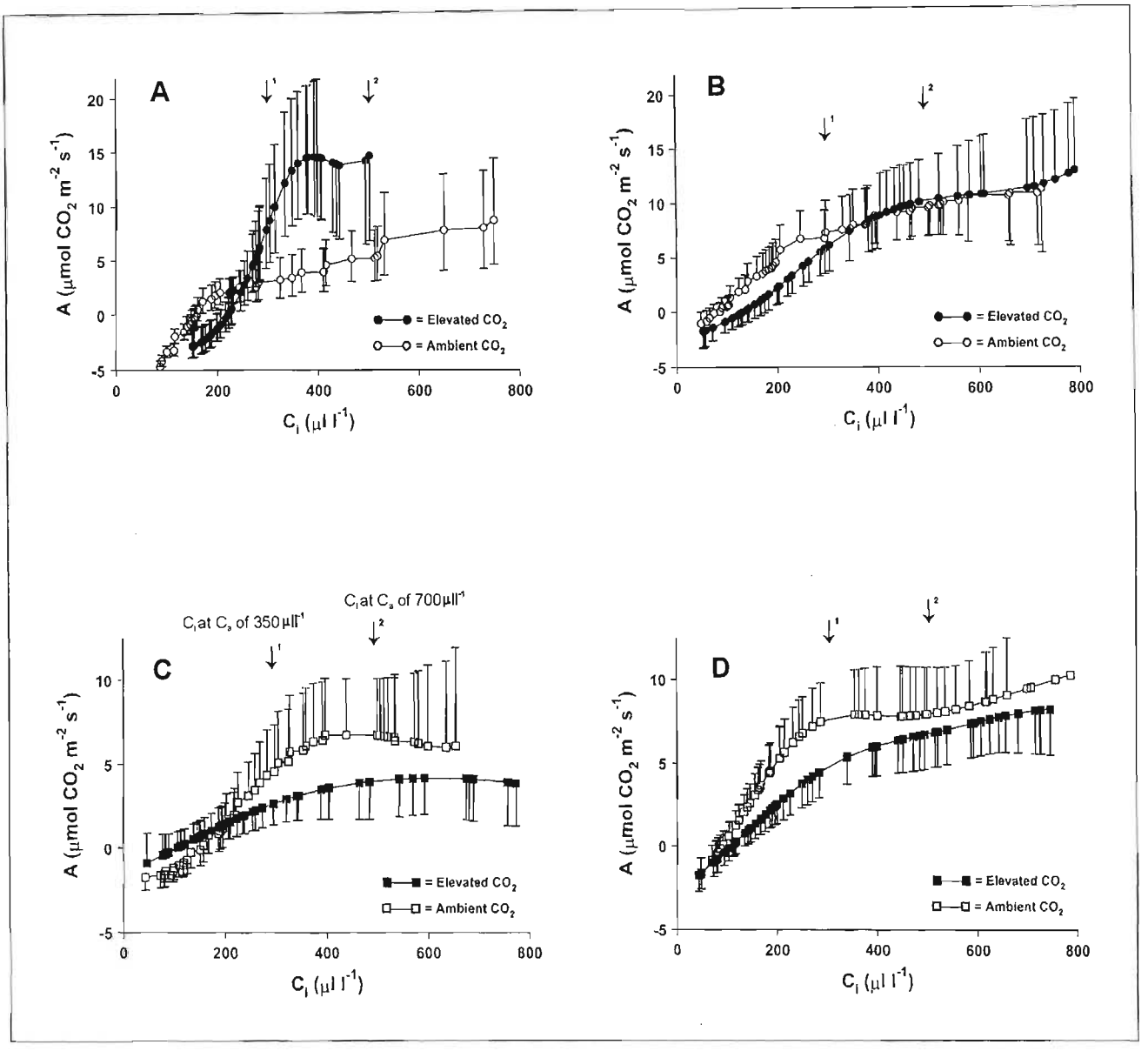

Figure 3.1. The effect of growing Acacias at an elevated (filled symbols) or ambient (open symbols) CO_2 level on the relationship between photosynthesis and internal CO_2 concentration (C_i). Where 'A' represents *Acacia nilotica* uninoculated; 'B', *A. nilotica* inoculated; 'C', *Acacia sieberana* uninoculated; and 'D', *A. sieberana* inoculated. $\downarrow^1$ = C_i at C_a of 350μll^{-1} (ambient) $\downarrow^2$ = C_i at C_a of approximately 700μll^{-1}.

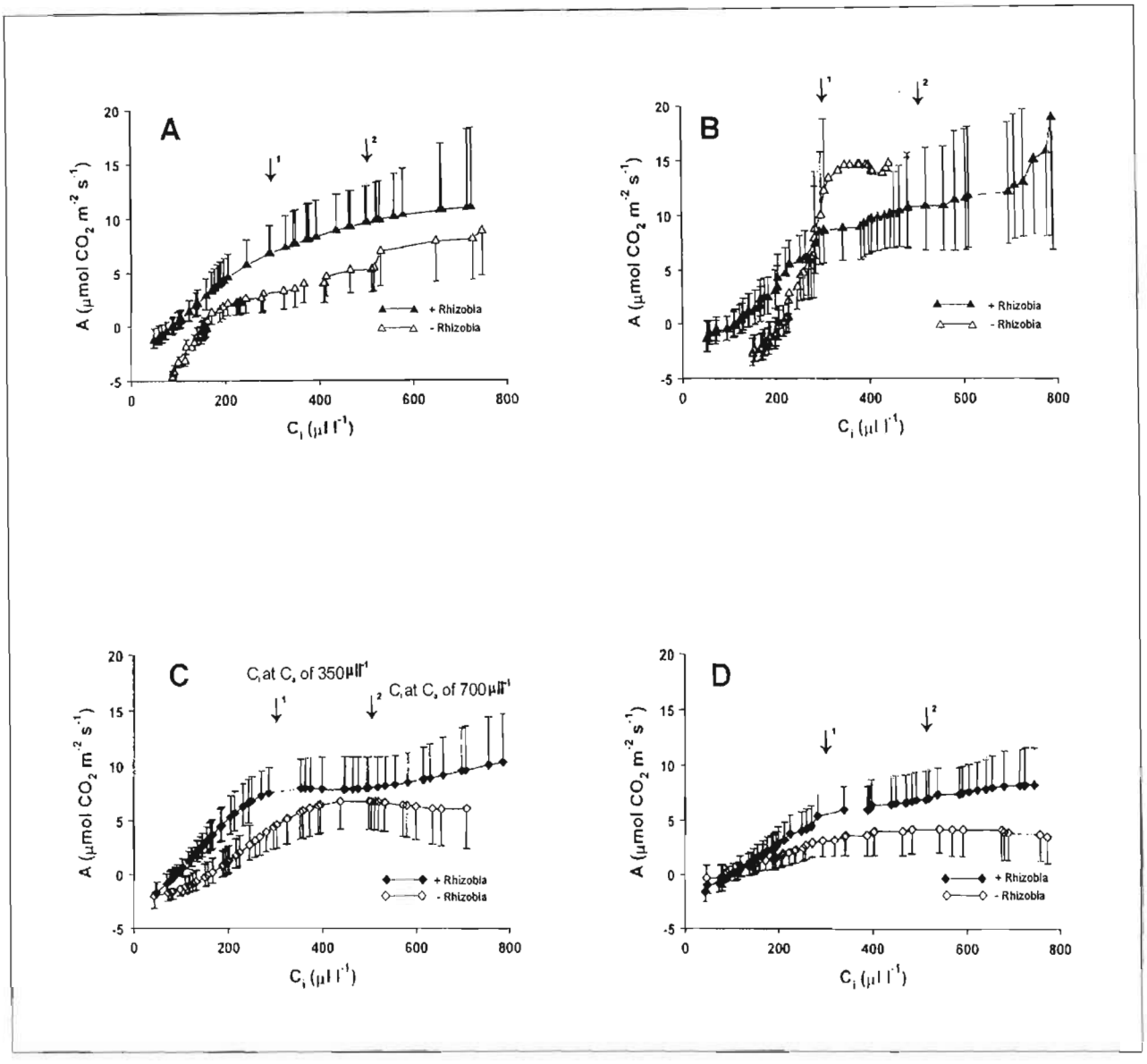

Figure 3.2. The effect of growing Acacias either with (filled symbols) or without (open symbols) rhizobial inoculation, in ambient or elevated CO_2, on the relationship between photosynthesis and internal CO_2 concentration. Where 'A' represents *Acacia nilotica* under ambient CO_2; 'B', *A. nilotica* elevated CO_2; 'C', *Acacia sieberana* ambient CO_2 and 'D', *A. sieberana* elevated CO_2. $\downarrow^1$ = C_i at C_a of 350μll^{-1} (ambient) $\downarrow^2$= C_i at C_a of approximately 700μll^{-1}

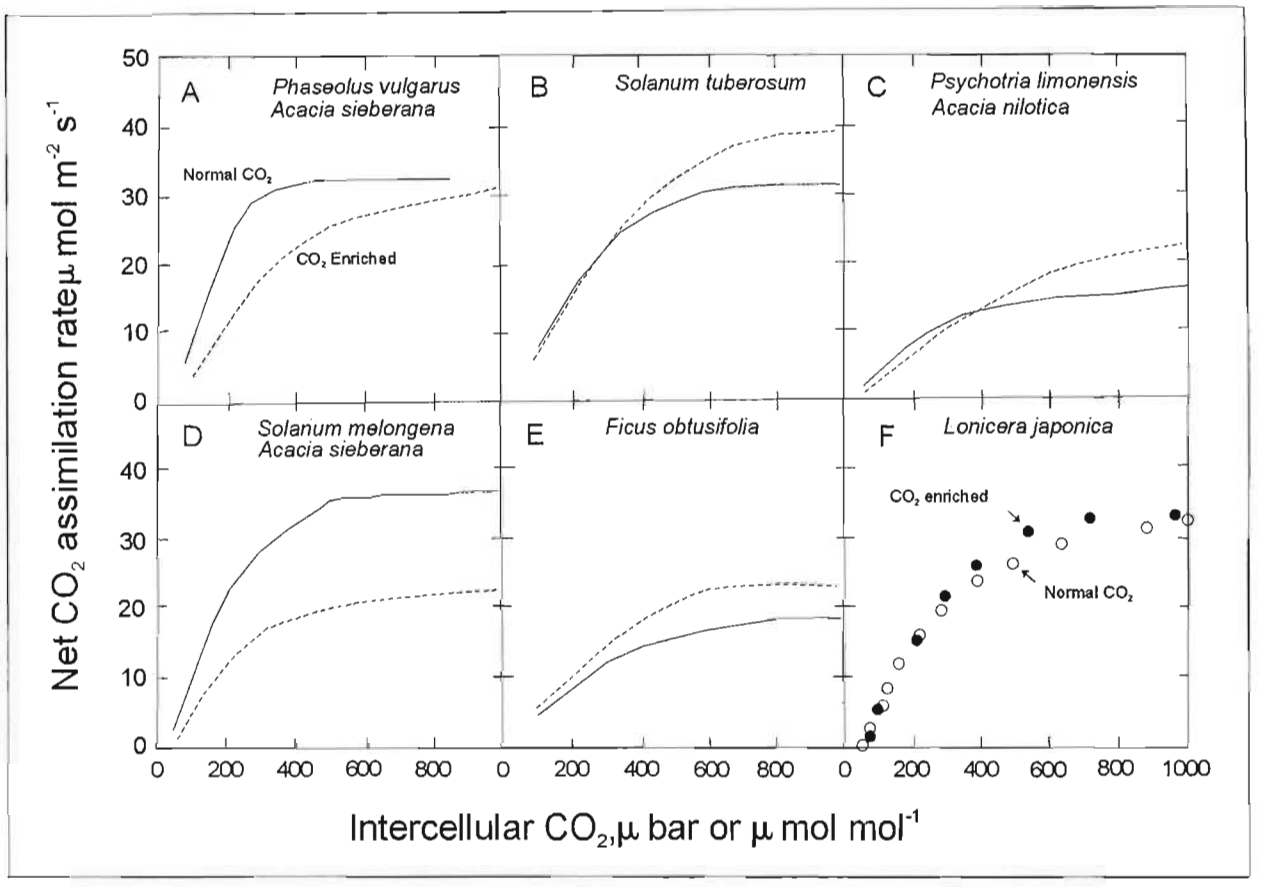

Figure 3.3. Examples of six A/C_i response patterns commonly observed in C_3 species grown in normal ambient (solid lines) or enriched (dashed lines) atmospheres of CO_2. Data from SAGE *et al.* 1989 (for *Solanum tuberosum* and *Phaseolus vulgaris*), ZISKA *et al.* 1991 (*Psychotria limonensis* and *Ficus obtusifolia*), SAGE unpublished (*Solanum melongena*), and REID & SAGE unpublished (*Lonicera japonica*). Figure redrawn and modified from SAGE 1994. Approximate *Acacia sieberana* and *Acacia nilotica* response patterns added to this figure from results obtained in the present study.

In *A. sieberana* the main separating factor between the treatments was the presence of rhizobia. Results from both ambient and elevated CO_2 plants inoculated with rhizobia were significantly different from the other two *A. sieberana* treatments, which were almost identical. Ambient-grown inoculated *A. sieberana* had the greatest stomatal conductance (Ave. stomatal conductance = 80.2 mol m^{-2} s^{-1}) followed by plants which were grown in elevated CO_2 and inoculated (Ave. stomatal conductance = 66.0 mol m^{-2} s^{-1}).

3.4 Discussion

Although there were differences, these were not significant for photosynthetic rates at any internal CO_2 concentration between plants grown in elevated CO_2 and those grown in ambient conditions. While the curves showed a difference, or even clear trends, there was constant overlap between the treatments. This overlap between the treatments is graphically illustrated in **Figures 3.1 and 3.2** (*N.B.* overlapping error bars have been erased for clarity). When photosynthesis was plotted against internal CO_2 concentration (A/C_i), the initial slopes of the graphs for both *A. sieberana* and *A. nilotica* were shallower for plants grown in elevated CO_2 compared to plants grown in ambient conditions, indicating a decreased Rubisco concentration and greater nitrogen use efficiency (**Figure 3.1**). At higher C_i *A. sieberana* had lower photosynthetic rates in plants grown at elevated CO_2 levels, suggesting an inability to regenerate RuBP or the possible accumulation of soluble carbohydrates. *A. nilotica* had a slightly increased P_i regeneration capacity when grown and measured at higher CO_2 concentrations, as compared to *A. nilotica* measured at elevated CO_2, but grown under ambient conditions.

When CO_2 enrichment increases plant size relative to the supply of nutrients, the availability of nutrients for photosynthesis can decline, reducing photosynthetic capacity (SAGE *et al.* 1989; SAGE 1994). Since plants maximise resource use efficiency by reallocating resources, nitrogen can be redistributed to maintain a balance between all components of the photosynthetic apparatus and between photosynthetic and non-photosynthetic processes (SAGE *et al.* 1989). Proportionally more N is transferred from non-limiting processes to those that limit A after CO_2 enhancement. Thus reduced photosynthetic rates may reflect either a reallocation of N away from Rubisco, into light harvesting, electron transport and P_i regeneration processes, or, a reallocation of N from

photosynthetic to non-photosynthetic processes (SAGE *et al.* 1989). A decrease in plant nitrogen concentration is often indicative of a decrease in the amount of Rubisco, although this was not reflected in the leaf nitrogen levels (see Chapter 4).

The two species in this study fitted into the following grouping (**Figure 3.3** [according to SAGE *et al* 1989; SAGE 1994]); in *A. nilotica* Rubisco capacity was reduced and P_i regeneration capacity increased under elevated CO_2. This is representative of the most efficient response (**Fig. 3.3A**). *A. sieberana* showed two separate responses. All photosynthetic components were reduced as a result of high growth C_a resulting in a lower A at all C_i values (**Fig 3.3D**) or growth at a high CO_2 level caused Rubisco and thylakoid dependent RuBP regeneration capacity to decline, without the P_i regeneration capacity being inhibited (**Fig 3.3A**). SAGE (1994) made a summary of over 30 studies, and found no pattern between the type of A/C_i response to long-term CO_2 enrichment, and the life form or ecological requirements of the species studied.

VOLIN and REICH (1996) found that the tendency for photosynthesis to be lower at elevated CO_2 was more pronounced in low, compared to high nitrogen, and root mass was significantly greater for plants grown in high nitrogen. In this study photosynthesis was decreased in plants grown without rhizobial inoculation (akin to low nitrogen) in both ambient and elevated CO_2, except for the *A. nilotica* uninoculated treatment (**Figure 3.2**).

A. sieberana had greater levels of stomatal conductance than *A. nilotica* and this was particularly pronounced when plants were inoculated, but decreased under elevated CO_2 (**Table 3.1**). This is a trend often-encountered (WAND *et al.* 1996). Because the relationship between A and stomatal conductance is non-linear in the range of C_i typically observed at high CO_2, a reduction in the C_i/C_a translates into a much larger reduction in stomatal conductance and a substantial increase in water use efficiency (WUE). This reduction in conductance may have important implications for the ability of droughted plants to survive in future warmer, high-CO_2 environments (SAGE 1994). C_i/C_a ratios were not compared in this study.

For *A. sieberana* there was an overall decrease in stomatal conductance when grown under elevated CO_2, but no accompanying significant increase in photosynthetic rate. The results for *A. nilotica* were somewhat distorted. *A. nilotica* grown with rhizobia showed a

slight increase in photosynthetic rates under elevated CO_2 as compared to ambient grown plants and lower stomatal conductance's than *A. sieberana*, but not much difference within the species between treatments (**Table 3.1**). The difference between the elevated CO_2 treatment (inoculated) and the other treatments was just significant however. Uninoculated *A. nilotica* demonstrated an abrupt increase in photosynthetic rates when exposed to elevated CO_2. This suggests that *A. nilotica* may be better able to withstand periods of prolonged drought than *A. sieberana*, irrespective of the CO_2 level and in the absence of rhizobial inoculation. WUE in *A. sieberana* as extrapolated from these stomatal conductance results would appear to be much less conducive to survival in the drier conditions, which will occur as a result of global warming.

In *A. nilotica* there were no significant differences between the treatments, apart from one anomalous result that could have been as a result of experimental error. In this case the plants grown under elevated CO_2 (uninoculated) had significantly lower stomatal conductances than all the other plants/treatments. Reductions in stomatal conductance and the associated increase in A resulting from CO_2 enrichment (the exact mechanism is unclear) are the principle factors increasing WUE at elevated C_a (EAMUS and JARVIS 1989; POORTER 1993; SAGE 1994).

3.5 Conclusions

There were no significant differences in photosynthetic rate (A) at any internal CO_2 concentration between plants grown in elevated CO_2 compared to those grown under ambient conditions. When photosynthesis was plotted against internal CO_2 concentration (A/C_i), the initial slopes of the graphs for both *A. sieberana* and *A. nilotica* were shallower for plants grown in elevated CO_2, compared to plants grown in ambient conditions. This indicated a decreased Rubisco concentration at low C_i, and greater nitrogen use efficiency. At higher C_i, *A. sieberana* continued to have lower A in plants grown at elevated CO_2 levels suggesting an inability to regenerate RuBP or the possible accumulation of soluble carbohydrates. *A. nilotica* grown in elevated CO_2 had a slightly increased P_i regeneration capacity at higher CO_2 concentrations. While the A/C_i results demonstrate that CO_2 has a minor effect on photosynthesis, growth responses indicated otherwise. Depressed photosynthetic rates under elevated CO_2 were not expected in this experiment, but a

reduction in stomatal conductance was expected. Thus while photosynthesis was unexpectedly decreased, this was offset by some other mechanism to result in a significant increase in plant mass under elevated CO_2 upon termination of the experiment (Chapter 2). A simple explanation for the increase in growth (but not photosynthesis) could be that dark respiration was less in plants grown at elevated CO_2 – for reasons that remain unclear.

Plants, even within species of close genera, will demonstrate differential responses to elevated CO_2 and so, plant community composition will change, resulting in changes in all other spheres as well.

While many of the expected results were attained in this study, some of the results demonstrated a distinct lack of pattern or explanation which may be ascribed to experimental error in some instances. This was unfortunate, and any further work in this field should take cognisance of this factor, which could possibly be resolved with more repetitions and early elimination of results that are obviously skewed.

CHAPTER 4

The effects of elevated CO_2 and rhizobial inoculation on *Acacia* leaf nitrogen concentration and possible implications for the future

4.1 Introduction

If elevated CO_2 concentrations promote growth, nitrogen demand in plants will increase. This may at least in part be offset by increased nitrogen use efficiency (COLEMAN and BAZZAZ 1992; LUO and MOONEY 1995) which may involve increased associations with mycorrhizae and N_2 fixers. According to the models, resources of abundant availability should be allocated to optimise the acquisition of the most limiting resources (CHAPIN *et al.* 1987; BASSIRIRAD *et al.* 1996; PRIOR *et al.* 1997). One may therefore expect nitrogen uptake rate per unit root mass and root growth to be positively affected by CO_2 enrichment (BASSIRIRAD *et al.* 1996) and aboveground biomass to be stimulated in plants grown in ambient CO_2, assuming that CO_2 is the most limiting resource (PRIOR *et al.* 1997). This idea was supported by the finding that water stressed trees grown in elevated CO_2 had a higher proportion of biomass in their roots (NORBY *et al.* 1986; HUNT *et al.* 1996; PRIOR *et al.* 1997). Reduced plant nitrogen concentration is commonly observed, (GARBUTT *et al.* 1990; BASSIRIRAD *et al.* 1996; CAMPBELL and HART 1996; MJWARA *et al.* 1996; WILSEY 1996) and may even be an effect of accelerated growth under elevated CO_2 (COLEMAN *et al.* 1993, cited in HUNT *et al.* 1996). Maximum root nitrogen uptake can also be severely depressed in response to elevated CO_2. This can occur because specific nitrogen sources and concentrations can dramatically affect subsequent nitrogen uptake, assimilation and partitioning in plants. Accumulation of reduced nitrogen in fine roots may therefore be responsible for the inhibitory effects of CO_2 on nitrogen uptake (BASSIRIRAD 1996).

It is of interest to compare the response of nitrogen fixing and non-nitrogen fixing species to elevated carbon dioxide. A nitrogen-fixing plant might be able to exploit an enriched CO_2 atmosphere more than a non-fixing or inactive nitrogen-fixing plant. Increased carbon in

terrestrial ecosystems in an elevated CO_2 environment will alter plant uptake, fixation and mineralization, leading to changes in ecosystem nitrogen dynamics (LUO and MOONEY 1995). Therefore, the distribution and presence of root nodule bacteria may play an important role in the welfare and effectiveness of CO_2 exploitation in an elevated CO_2 environment for these plants in the future. Thus, increases in atmospheric CO_2 may enhance the spatial variability of plants.

In C_3 plants photosynthesis is always accompanied by photorespiration, a wasteful process which consumes oxygen and releases CO_2 in the presence of light. Photorespiration is not accompanied by oxidative phosphorylation and therefore yields no ATP. Furthermore, photorespiration diverts some of the reducing power generated in the light reactions from the biosynthesis of glucose into the reduction of oxygen. Under normal atmospheric conditions as much as 50 percent of the carbon fixed in photosynthesis by a C_3 plant may be reoxidized to CO_2 during photorespiration. Photorespiration is almost completely absent in C_4 plants. Glycolic acid is the major substrate oxidised during photorespiration, by the oxidative breakdown of RuBP by RuBP carboxylase. RuBP carboxylase can promote the reaction of RuBP with either CO_2 or O_2. When the CO_2 concentration is high and that of O_2 is relatively low, RuBP carboxylase fixes CO_2 to RuBP to yield 3-phosphoglycerate (PGA (two molecules)). When the CO_2 concentration is low and that of O_2 is relatively high, the enzyme acts as an oxygenase, combining RuBP and O_2 to yield phosphoglycolic acid and PGA. The phosphoglycolic acid is then converted to glycolic acid - the substrate oxidised during photorespiration. High CO_2 and low O_2 concentrations limit photorespiration, as a result plants need less Rubisco. C_4 plants have a distinct advantage over C_3 plants because CO_2 fixed by the C_4 pathway is maintained and keeps a high CO_2:O_2 ratio at the site of the action of RuBP carboxylase. This favours the carboxylation of RuBP. When photorespiration is reduced (i.e. under high CO_2 and low O_2) the enzymes and nitrogen ordinarily tied up in the photorespiratory cycle become available to the plant, but are then not needed as much.

The nitrogen cycle is complex because of the many natural compounds that nitrogen can form, the number of transformations between these compounds, and the influence of complex environmental and biological factors on their formation. Nitrogen occurs both as a gas in the atmosphere and bound in the earth in sedimentary and primary rocks (POSTGATE 1978). Nitrogen locked up in rocks in the earth's crust is generally not available

to biological processes. Nitrogen occurring in the atmosphere is dinitrogen gas (N_2) and forms 79.08% of the atmosphere (STEVENSON 1965). The atmosphere is the most available source of nitrogen for assimilation by organisms. Atmospheric nitrogen must be converted to inorganic nitrogen, either ammonia (NH_3) or nitrate (NO_3). Only then can most living organisms assimilate it. To compound the problem, N_2 is an inert gas and as such does not readily form compounds in nature. Only a few organisms (some prokaryotes) can use elemental nitrogen (by nitrogen fixation). All other organisms must obtain their nitrogen either directly or indirectly as combined nitrogen.

The process of converting N_2 gas to inorganic nitrogen compounds is called nitrogen fixation. Assimilation is the term applied to the biological conversion of inorganic nitrogen to organic nitrogen such as amino acids and protein (POSTGATE 1978). Uptake refers to the gathering of nitrates and ammonium by plant roots and must occur before assimilation can occur. Nitrogen is cycled between the atmosphere, soil, soil microfauna, plants and animals. SWITZER and NELSON (1972) defined the cycling processes in forest ecosystems in terms of (a) the biogeochemical cycle between the plant and the soil, (b) the biochemical cycle of internal transfer within the plant and (c) the geochemical cycle of import-export. WOODMANSEE (1978) separated the cycling process into biological cycles, geological cycles and meteorological cycles. He described three linking vectors between these cycles - water, atmosphere (gases, aerosols, and particulates), and animals.

The aim of this experiment was to extrapolate whether variability in nitrogen distribution (represented by rhizobial inoculation in nature and in this experiment) would affect the growth and distribution of *Acacia* species under future elevated CO_2 conditions. In naturally occurring Acacias, not all individuals are infected by *Rhizobia* sp., as this is most often dependant on the availability of rhizobial inoculums in the soil. Thus only some plants will benefit from improved nitrogen uptake, or fixation from the air. If plants are to extend into new ranges as a result of elevated CO_2 and global warming, they may encounter soils previously uninfected by *Rhizobia*, and hence, responses and competition effects will be different to those encountered within their current distributional limits. It was these differences in response, to elevated CO_2 and rhizobial inoculation between two *Acacia* species that this experiment hoped to elucidate.

4.2 Materials and Methods

The plants used (in the previous experiments) for growth and photosynthetic analysis were also used for nitrogen analysis. The saplings were grown in this way over a period of seven months during which time growth rates (change in height and height at harvest) and photosynthetic rates were monitored. Root nodule formation was determined by visual inspection. All the plants that were initially inoculated were observed to be nodulated at the final harvest. When the 'uninoculated' plants became 'infected' with rhizobial root bacteria towards the end of the experiment, the experiment was terminated. Leaf nitrogen content was determined on oven-dried leaves, using micro-Kjeldahl digestion (Buchi 430 digestor) followed by steam distillation (Buchi 321 distillation unit) and titration of the ammonium collected.

For each sample the dried crushed leaves of two plants from each treatment were combined. The plant material was placed in an oven at 80°C and left until it reached constant weight. The plant material was then placed in a desiccator to cool before weighing. One gram of the crushed leaves from the randomly selected plants was used. This sample was added to the digestion flasks, together with 3 glass beads and one Kjeldahl tablet, then 20 ml of concentrated sulphuric acid added. A blank was also always used. The flasks were then connected to the extraction tubes in the digestion unit, which was preheated for 5 -10 minutes before use. The time required for digestion was between one to two hours, each digestion being allowed to run until the solution was transparent (light green or yellow). When the digestion was complete, the tubes were removed and allowed to cool. The samples were then distilled, but before each distillation a blank distillation was performed. The samples were diluted with three parts water per one part sample, 100ml of 30% NaOH was then added to each sample to neutralise it. An Ehrlenmeyer flask containing 100ml of 2% boric acid and 0.2ml bromocresol green indicator was placed underneath each distillation outlet. Approximately 150ml were distilled over 5 - 10 minutes. These samples were then titrated against standardised 0.1M HCl.

Percentage nitrogen and protein were calculated as follows:

% Nitrogen = (volume added x molarity of the acid x 1.401) / weight of the sample.

% Protein = % nitrogen x 6.25

Ten to fourteen repetitions for each of the eight treatments were conducted (14 when the deviation between individual treatment results seemed large).

4.3 Results

Tables 4.1, 4.2, and 4.3 summarise the analyses of leaf nitrogen in the two *Acacia* species subject to the various treatments. *A. nilotica* had the largest difference in nitrogen content between treatments, but the lowest percentage leaf nitrogen ($\bar{x}$ ± S.E. = 1.45 ± 0.19%). *A. sieberana* had the highest percentage leaf nitrogen ($\bar{x}$ ± S.E. = 1.89 ± 0.27%) with small insignificant differences between the treatments.

Table 4.1. The effect of CO_2 level and rhizobial inoculation on leaf nitrogen in *Acacia sieberana* and *Acacia nilotica*. Values given are means across all treatments (* $P < 0.005$, ** $P < 0.001$).

Factor	Leaf N (%)	Leaf protein (%)	Significance
Species			
A. sieberana	1.89	11.81	
A. nilotica	1.45	9.06	**
CO_2 level			
elevated	1.69	10.56	
ambient	1.65	10.31	n.s.
Rhizobial inoculation			
Inoculated	1.74	10.87	
Uninoculated	1.59	9.94	.*

The CO_2 level had the least effect on percentage leaf nitrogen (**Tables 4.1 and 4.2**). Contrary to expectations plants of both species grown in elevated CO_2 did not have a significantly lower leaf nitrogen *percentage* than those grown in ambient CO_2 ($P = 0.418$), although nitrogen *uptake* was significantly greater for plants grown in elevated CO_2 and with rhizobial inoculation (**Table 4.3**). Multiple ANOVA indicated that the presence/absence of rhizobial inoculation was significant ($P < 0.005$) between both species over the two CO_2 levels (i.e. all other factors were combined, and presence/absence of significant differences in rhizobial inoculation determined). Inoculated plants had a higher percentage of leaf nitrogen and greater nitrogen uptake than uninoculated plants (**Tables 4.1 and 4.3**). When each species was analysed individually it was found that there was no significant difference in *A. sieberana* leaf nitrogen

percentage with respect to either CO_2 level or rhizobial inoculation or the interaction of these two factors (**Table 4.2**). However, analysis of *A. nilotica* leaf nitrogen percentage data revealed that there was a highly significant difference in the inoculation treatments ($P < 0.0001$), but no difference between CO_2 level treatments. The interaction of the CO_2 level x inoculation treatment was significant ($P < 0.005$) in *A. nilotica*.

Table 4.2. Effects of CO_2 level (elevated/ambient), and rhizobial inoculation (inoculated/uninoculated) and the interaction of the two (CO_2 level and rhizobial inoculation), on *Acacia sieberana* and *Acacia nilotica* leaf nitrogen, calculated separately for each species (* $P < 0.005$, ** $P < 0.001$).

Factor	Significance
Acacia sieberana	
CO_2 level	n.s.
Rhizobial inoculation	n.s.
CO_2 x inoculation	n.s.
Acacia nilotica	
CO_2 level	n.s.
Rhizobial inoculation	**
CO_2 x inoculation	.*

Table 4.3. The effects of CO_2 level (elevated/ambient) and rhizobial inoculation (inoculated/uninoculated) on leaf nitrogen (%) and total leaf uptake (% per g) in *A. sieberana* and *A. nilotica*. Figures followed by the same letter are not significantly different ($P < 0.05$).

Treatment	Leaf N (%)	Nitrogen uptake (% per g)
A. sieberana		
elevated CO_2, inoculated	1.9 ± 0.3 ^{d}	7.22 ^{c}
elevated CO_2, uninoculated	1.9 ± 0.3 ^{cd}	5.05 ^{b}
ambient CO_2, inoculated	1.9 ± 0.2 ^{cd}	6.50 ^{b,c}
ambient CO_2, uninoculated	1.8 ± 0.3 ^{cd}	3.13 ^{a}
A. nilotica		
elevated CO_2, inoculated	1.5 ± 0.1 ^{ab}	6.30 ^{c}
elevated CO_2, uninoculated	1.4 ± 0.2 ^{ab}	3.09 ^{a,b}
ambient CO_2, inoculated	1.6 ± 0.1 ^{bc}	5.30 ^{b}
ambient CO_2, uninoculated	1.3 ± 0.2 ^{a}	1.94 ^{a}

4.4 Discussion

Nitrogen concentration increased by a small (2%) percentage in *A. sieberana* and *A. nilotica* saplings (**Table 4.1**) grown under elevated CO_2. However, despite the lack of a

large (or highly significant) response to elevated CO_2, (**Table 4.2**) the nitrogen concentration was still enhanced at high CO_2 concentrations, although differently in each species (**Table 4.3**). Nitrogen uptake was significantly enhanced under elevated CO_2 and in inoculated plants. The relative increase in nitrogen concentration and uptake was markedly higher in *A. sieberana*, especially in inoculated plants. In *A. nilotica* the CO_2 level had less of an effect on nitrogen assimilation than inoculation, and the combination of inoculation and CO_2 (**Table 4.2 and Table 4.3**). Uptake and assimilation of carbon and nitrogen in plants are interrelated and changes in the availability or acquisition of one often leads to changes in availability and acquisition of the other. Plants grown at elevated CO_2 levels may have more carbohydrates available for the support of mycorrhizal or N-fixing bacteria, improving their nutrient status (BAZZAZ and MCCONNAUGHAY 1992). Leaf area expansion, growth and photosynthesis are all expected to increase under elevated CO_2 when combined with high nutrient levels, especially nitrogen (LUO and MOONEY 1995; BASSIRIRAD *et al.* 1996; VOLIN and REICH 1996).

BASSIRIRAD *et al.* (1996) found that elevated CO_2 increased (20 - 40%) total ^{15}N acquisition in pine species. They found that root uptake of ammonium (NH^{4+}) was severely depressed in response to elevated CO_2 in two species of pine. Nitrogen sources and concentrations can dramatically affect nitrogen uptake, assimilation and partitioning in plants. Increased soil exploration (DAY *et al.* 1996) and nitrogen uptake are the major mechanisms by which seedlings are able to respond to elevated CO_2 under nitrogen / nutrient limitations. It is suspected that the availability of nutrients in this study may have limited nutrient uptake and simultaneously reduced photosynthetic response. The accumulation of nitrogen in fine roots may be responsible for the inhibitory effects of CO_2 on nitrogen uptake (BASSIRIRAD *et al.* 1996). This occurs when elevated CO_2 causes significant increases in the N pool of fine roots, and significantly decreases the shoot N pool. BASSIRIRAD *et al.* (1996) showed that CO_2 enrichment significantly inhibited N transport capacity to the shoot, but were unable to determine if this response was caused by a change in root transport properties *per se* or by changes in shoot demand. The results of the present study were however contrary to expectations, in that plants grown in elevated CO_2 did not have significantly lower leaf nitrogen percentages when compared to plants grown in ambient CO_2. Average values noted in other studies include (means): 6.04% and 7.17% in low N, under ambient and elevated CO_2 respectively, and 5.22% and 5.59% in high N, under ambient and elevated CO_2 respectively (*Trifolium repens* (stolons), ZANETTI *et al.* 1996); and 1.61% and

1.14% in low N under ambient and elevated CO_2 respectively, and 2.57% and 2.40% in high N under ambient and elevated CO_2 respectively (*Pinus taeda* (leaves) GRIFFEN *et al.* 1993). While these reductions may be induced by elevated CO_2, acquisition responses to elevated CO_2 may largely depend on substrate N concentration or N form or both. More accurate prediction of plant and ecosystem responses to elevated CO_2 should include consideration of other factors that may affect nutrient acquisition. For example, the acquisition of relatively immobile ions such as phosphorus can be substantially facilitated by increased association with symbiotic rhizobia (BASSIRIRAD *et al.* 1996).

Nitrogen concentrations in the above ground plant material is usually reduced under elevated CO_2. This is attributed to carbon-assimilated-accumulation in leaves and / or an improvement in N efficiency under elevated CO_2. The reduction in N concentration appears to be less pronounced in legumes than in other grassland species (ZANETTI *et al.* 1996). The availability of N in the soil affects the performance of N_2 fixation in legumes. A low supply of N from fertilizer, as well as the presence of associated non-symbiotic plants competing for N, decrease the soil N availability, which is positively correlated with the percentage of N fixed symbiotically in many legume species (ZANETTI *et al.* 1996).

It may be that plants maximise resource use efficiency by allocating resources, mainly N, to maintain a balance between all components of the photosynthetic apparatus and between photosynthetic and non-photosynthetic processes (SAGE *et al.* 1989). This means that proportionally more N will be transferred from non-limiting processes to those which limit A after CO_2 enhancement. Thus reduced photosynthetic rates (and N concentrations) may reflect either a reallocation of N away from Rubisco and into light harvesting, electron transport and P_i regeneration processes, or reallocation of N from photosynthetic to non photosynthetic processes. Since Rubisco constitutes the single largest sink for N in the photosynthetic apparatus, changes in its content will have the greatest effect on N partitioning within the leaf (SAGE *et al.* 1989). It is therefore unfortunate that nitrogen concentrations in roots and in intact root systems were not determined in this study. This should be considered a limitation of the present study. Much of the plant nitrogen may have become concentrated in the roots of these plants.

In general, apart from the N supply (quality and quantity) and the presence of non-fixing associated plant species, other processes such as leaching, mineralization, denitrification,

and N immobilization influence the amount of N available to a plant and therefore to symbiotic fixation (ZANETTI *et al.* 1996). Under elevated CO_2, increases in N fixed by root nodules, as well as the total quantity of the C:N ratio of litter and root material, may alter below ground processes involved in nutrient cycles. Along with an increase in N immobilization into the expanded microbial biomass, enhanced denitrification may also reduce N availability (ZANETTI *et al.* 1996). Higher soil moisture (which was evident in this study) resulting from the lowered water use of plants exposed to elevated CO_2 along with elevated oxygen consumption by the increased microbial activity and root biomass (JONGEN *et al.* 1995, cited in ZANETTI *et al.* 1996), may lower the oxygen partial pressure in the soil and, therefore, favour denitrification activity (ZANETTI *et al.* 1996). High leaf nitrogen concentrations indicate that nutrient limitation is an unlikely factor. However, in this study, the leaf nitrogen concentrations were relatively low in all treatment combinations. This may indicate that the nutrient level was a limiting factor. Although the plants looked healthy, and nutrients were supplied according to the standard Hoagland's recipe (**Table 2.1**, Chapter 2), nitrogen was removed from the 'recipe' for many of the feedings. This may have contributed to the low nitrogen concentration upon measurement. The flow of water (and subsequently nutrients) through the sandy growth medium, which offers little resistance to flow, may also have been a contributing factor preventing adequate nutrient uptake. The nutrient concentration (fed to the plants) itself could have been too low, but this is unlikely, as these values were meticulously calculated and applied.

BASSIRIRAD *et al.* (1996) also found that despite the repression of nitrogen absorption capacity, actual uptake rates estimated on intact root systems were not significantly affected by CO_2. They found that CO_2 enrichment increased fine root ratio and total plant nitrogen uptake to a similar extent. Fine root ratios were not determined in the present study. SAGE *et al.* (1989) found increased, decreased and unchanged levels of nitrogen in their leaf N experimental data between five C_3 species. The lack of a consistent pattern is common place. With plant growth response being dependent on the edaphic conditions, the greater the availability of water and nutrients, the greater will be the growth enhancement with elevated CO_2 (BAZZAZ 1990; MUELLER-DOMBOIS 1992). Therefore, adjustments in the distribution of the nutrient pool within the plant or in the metabolic requirements could increase nutrient use efficiency. For example, if the efficiency of Rubisco is higher under elevated CO_2, less N would be needed for increased growth

(NORBY *et al.* 1986). Thus this additional N would be available within the plant, and N concentration would not decrease under elevated CO_2 conditions. Since high CO_2 limits photorespiration, and less Rubisco is required for plant functioning as a result, it may be possible that the increased photosynthetic rates (A) observed under elevated CO_2 in this study, were accompanied by additional N which was not required under the reduced photorespiration regime. Hence the lack of significant or large differences in N concentration across all the treatments. The data suggest that N was not a factor that limited plant growth (although inoculation increased growth). While elevated CO_2 concentrations increased growth, N concentration was not 'diluted' by the increased growth. The A/C_i analyses (Chapter 3) suggest that plants grown under elevated CO_2 concentrations should have had lower leaf N concentrations, but when measured they were found not to have. Possibly, inoculation increased growth by some way other than increasing N supply. It is suggested that light (and not N supply) limited growth.

MITCHELL *et al.* (1995) found that nitrogen supply strongly influenced respiration rate per unit leaf mass, per unit leaf area, and per unit foliar nitrogen content (*Pinus palustris*). Long-term atmospheric elevated CO_2 exposure resulted in greater respiration rates, per unit foliar nitrogen, and this effect was enhanced by the high nitrogen treatment as compared to low nitrogen treatment. Non-structural carbohydrates were increased by elevated CO_2 and decreased by increased nitrogen availability. Therefore the effects of CO_2 concentration on leaf growth respiration may be mainly due to increased growth rates rather than changes in the cost of construction. In addition to CO_2 concentration, nitrogen influenced leaf construction costs. These results were in agreement with those of GRIFFEN *et al.* (1993) who suggested that with elevated CO_2 and low nitrogen, the growth of some plants may be sink limited, resulting in an increase in non-structural carbohydrates. With elevated CO_2 and high nitrogen, plant growth (GRIFFEN *et al.* 1993, MITCHELL *et al.* 1995) may be source limited. The crux of the matter is this: nitrogen supply had a greater effect on both maintenance respiration and cost of constructing leaf tissue than did atmospheric CO_2 (MITCHELL *et al.* 1995).

Interestingly, MIDGLEY *et al.* (1995) reported that under lower nutrient supply rates, species associated with nutrient poor sands achieved greater biomass yield than those associated with more nutrient-rich soils. They suspected that this was due to greater reserves of nitrogen and phosphorous in the seed of species from nutrient poor sands. The data

presented in this study reiterates the importance of nitrogen and nitrogen fixation in these *Acacia* species, as all factors could be separated on the basis of the nitrogen status (in terms of rhizobial inoculation) of the plants. However, while nitrogen availability will be important in determining plant success in a future CO_2 enriched environment, it is unlikely that nitrogen limitations will preclude growth responses to elevated CO_2. Reiterating this point, STOCK *et al.* (1990, cited in MIDGLEY *et al.* 1995) suggested that South African and Australian plants, specifically Proteaceae, growing on acid sands, have developed a greater independence from soil nitrogen and phosphorus availability in seedling development. This is evidently as a result of nutrient status being a strong selective pressure on seed quality. It is unlikely that this degree of independence from edaphic conditions will develop in KwaZulu-Natal grasslands and savannas in the foreseeable future.

In a future elevated CO_2 environment herbivorous animals may be negatively affected if biomass production remains unchanged and percentage nitrogen decreases. Carbon: Nitrogen ratios in plant parts are predicted to increase and thereby decrease the nutritional value of foliage. It has been predicted that herbivorous animals may be forced to consume larger amounts of vegetation in order to meet their nutritional requirements, the ramifications of which are many and complex. The present study indicates that this decrease in nutritional value (in Acacias especially) in terms of nitrogen concentration may not occur as markedly as depicted by other authors in other species.

4.5 Conclusions

Permanent acclimation does not occur in plants well supplied with nitrogen when these are exposed to elevated CO_2. Any downward regulation which may occur, is due to both reduced RuBP saturated and RuBP limited photosynthetic capacity. The results obtained in this study suggest that the levels of nitrogen fertilization will become more important in determining growth and yield potentials, but possibly not nutrient values (carbon:nitrogen ratios in leaves, for instance) of plants under conditions of climate change.

The relative contribution of changes in root growth versus physiological uptake capacity in determining nitrogen acquisition responses to elevated CO_2 may largely depend on nitrogen concentration or nitrogen form or both. If soil available nitrogen is relatively low

and is dominated by ammonium ions (NH^{4+}), compensatory changes in root growth may be the most important root characteristic determining plant nitrogen uptake response to high CO_2 (root shape/type will change with different types of nitrogen) (BASSIRIRAD *et al.* 1996). A more accurate prediction of plant and ecosystem response to elevated CO_2 must include other factors that may affect nutrient acquisition. Mycorrhizae and root nodule bacteria will therefore also play a crucial role in regulating plant nitrogen uptake in many species, and should therefore also be considered when examining changes in plant nutrient acquisition in response to CO_2 enrichment (BASSIRIRAD *et al.* 1996).

This study indicates that nitrogen availability in terms of rhizobial inoculation/ availability will be important in determining future dispersal and distribution patterns of Acacias like *A. sieberana* and *A. nilotica*, particularly in terms of biomass acquisition as described earlier (Chapter 2). With the increases in CO_2 and associated increases in temperature, areas in which root nodule bacteria are present in the soil may experience greater invasions of *Acacia* species than areas in which the root nodule bacteria do not proliferate. For instance, the Cathedral Peak area in the Drakensberg may be particularly well suited to *Acacia* growth, especially *A. sieberana*, (Chapter 5) or it may be that *A. sieberana* is more of a r-species invader, faring well upon initial establishment.

CHAPTER 5

The responses of a C_3 tree seedling (*Acacia sieberana*) when grown with one of two grasses (either C_3 or C_4)

5.1 Introduction

Assuming that environments enriched with atmospheric CO_2 are resource-rich, plant - plant interactions should increase under elevated CO_2. ARNONE (1996) was of the opinion that the most reliable prediction for a CO_2 rich world, was that there would be some level of shifts in species dominance. He indicated that these shifts would occur rapidly in nutrient-rich systems containing young plants and more slowly in nutrient-poor systems or those containing older plants. Responses of individually grown plants to high CO_2 do not scale well (or convert) to those obtained when individuals are grown in competitive situations (BAZZAZ and MCCONNAUGHAY 1992; ROGERS and DAHLMAN 1993; ELLSWORTH *et al.* 1995; ARNONE 1996; STEWART and POTVIN 1996). There are innumerable positive and negative feedback's occurring at all levels in an ecosystem, the complexity of which will influence the structure and composition of plant communities. It is only possible at this stage to say, with any degree of certainty, that elevated CO_2 will lead to changes in species dominance. Eventually this will alter patterns of plant succession and vegetation cover (ARCHER 1990; BAZZAZ 1990; RAWSON 1992; ARNONE 1996; PRIOR *et al.* 1997).

STEWART and POTVIN (1996) found that competition, net invasions, and total invasions all increased with enriched CO_2. This effect of CO_2 on invasiveness was species specific and benefited C_3 plants more than C_4 plants. Species may be able to co-exist by the following mechanisms. Co-existence may be possible for species with large niche differences or conversely, species with similar competitive ability could co-exist because competitive exclusion could not operate. Competition among neighbouring plants can be said to operate if one of the plants alters the availability and/or quality of some resource or condition in the shared environment. Secondly, its neighbour must perceive the environmental change *and* grow more poorly under these altered conditions (BAZZAZ and MCCONNAUGHAY 1992). This negative interaction need not be reciprocal and usually is not.

To determine the possible effects of competition between the acacias and typical competitors as found in savannas (C_3 and C_4 grasses), a competition experiment was initiated. The main component or 'token' species of grasslands and savannas are the acacias and grass species. For this reason it was decided to grow one of the acacias, (*Acacia sieberana*) with one of two grasses, *Dactylus glomerata* (a C_3 grass) and *Eragrostis curvula* var. Ermelo (C_4). Differences in growth (mass) of these grasses under elevated CO_2, and the competitive effects that might become apparent when grown with the C_3 *Acacia* were evaluated.

5.2 Materials and Methods

The same growth chambers and growing conditions were used as in the earlier experiments (Chapters 1 - 4). However, in this experiment, *Acacia sieberana* was either grown alone, or with four plants of either *D. glomerata* or *E. curvula*. Within each CO_2 concentration (ambient / elevated) treatment 10 pots of each combination (*Acacia* and grass) were inoculated with *Rhizobium* sp. and 10 pots were left uninoculated. Ten pots of four plants of each of the grasses were also grown alone in both of the chambers (totalling 80 pots per chamber). Those pots that were left uninoculated were grown on upturned plant pot bases. This was to avoid as much 'contamination' by the rhizobially inoculated plants as possible, a problem which had arisen in the earlier experiment, and which seemed to be as a result of water-borne rhizobia species. This modification in the experimental protocol had the desired effect, and by the end of the experiment, only those plants initially inoculated were still inoculated at harvest.

Light intensity (solar radiation through the greenhouse and the experimental chamber) was between 550 and 350 µmol m^{-2} s^{-1} at the soil surface. This light flux was certainly lower than that encountered in the field on a sunny day (1580 - 510 µmol m^{-2} s^{-1}). This might be considered a particular disadvantage for the C_4 plant, since C_4 fixation requires more energy in the form of ATP than does C_3 fixation. Photosynthesis of C_4 plants typically saturates at higher light levels than C_3 plants (HUNT *et al.* 1996).

The experiment was terminated after 6 months, once the *Acacia* and grass masses had reached unmanageable proportions. Only above ground mass was determined for each plant as the root masses were too intermingled to effectively separate without significantly

influencing the results. The plants were initially weighed upon harvesting and then dried at 75-80°C for 48 hours, or until equilibrium was reached, and then re-weighed. The differences in the mass of fresh and dry matter of *A. sieberana* , *D. glomerata* and *E. curvula* were analysed using a Multifactor Analysis of Variance (MANOVA).

5.3 Results

Comparisons between CO_2, rhizobia, and the presence/absence of grass treatments revealed no significant differences between the CO_2 treatments. A slight difference in favour of inoculated plants was found in the rhizobial treatments, and a significant difference depending on the presence or absence of a grass ($P < 0.001$, **Table 5.1**). In the presence of grass the *Acacia* fresh and dry masses were reduced. When the total *Acacia* mass was compared across the range of treatments (CO_2 level, rhizobial inoculation, and grass presence/absence), CO_2 fertilization had the least effect and the presence of a grass, the greatest effect, on plant mass.

Table 5.1. Effect of a competing grass species, CO_2 level (elevated / ambient) and rhizobial inoculation on total *A. sieberana* dry and fresh masses ± std deviation. (* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$, n.s. not significant). n = number of replicates.

Factor	*A. sieberana* fresh mass (g)	*P*	*A. sieberana* dry mass (g)	*P*	n
A. Grass species					
None	8.9 ± 2.7		2.3 ± 0.6		40
C_3 grass	2.2 ± 1.1	***	0.7 ± 0.3	***	40
C_4 grass	7.2 ± 2.8		1.9 ± 0.7		40
B. CO_2 level					
Elevated	6.4 ± 4.1		1.7 ± 1.1		60
Ambient	5.8 ± 3.1	n.s.	1.6 ± 0.8	n.s.	60
C. Rhizobial inoculation					
Inoculated	6.5 ± 3.9		1.7 ± 1.0		60
Uninoculated	5.8 ± 3.3	n.s.	1.5 ± 0.8	*	60
Interactions					
AB Grass species x CO_2 level		**		**	
AC Grass species x inoculation		n.s		n.s	
BC CO_2 level x inoculation		n.s		n.s	

When the individual effects of each treatment on *Acacia* fresh and dry mass are considered, only the presence of a grass, and the particular species is significant ($P < 0.001$). CO_2 concentration and rhizobial inoculation (except dry mass) analysed in isolation of the other treatments are not significant (**Tables 5.1 and 5.3**).

Table 5.2. Effect of a competing tree seedling (*A. sieberana*), CO_2 level (elevated / ambient) and rhizobial inoculation on total grass dry and fresh masses ± std deviation. (* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$, n.s. not significant).

Factor	Grass fresh mass (g)	*P*	Grass dry mass (g)	*P*	n
Grass species					
C_3 grass	10.3 ± 1.9		2.7 ± 5.0		40
C_4 grass	4.1 ± 1.8	***	1.1 ± 0.6	*	40
Presence of tree seedling					
Present	6.8 ± 3.9		1.9 ± 4.4		40
Absent	8.0 ± 2.7	n.s. (0.06)	1.8 ± 0.5	n.s.	40
CO_2 level					
Elevated	7.6 ± 4.1		1.8 ± 0.9		40
Ambient	6.8 ± 2.9	n.s.	2.0 ± 5.1	n.s.	40
Rhizobial inoculation					
Inoculated	7.3 ± 4.0		1.6 ± 0.9		40
Uninoculated	7.1 ± 3.4	n.s	2.1 ± 4.4	n.s.	40

When the masses of both grass species are averaged and analysed for each treatment individually, again only the grass species treatment was significant. The C_3 grass accumulated much more mass than the C_4 species ($P < 0.001$). Although the absence of the tree seedling and elevated CO_2 increased grass mass, this was not significant (**Tables 5.2 and 5.3**). C_4 grass mass was always greater under ambient CO_2 conditions (**Table 5.4**), which may have served to reduce the significance of the effect of CO_2 on grass mass as a group.

When the differences in mass in the specific grass species were analysed, it was established that the C_3 species, *D. glomerata*, accumulated significantly more mass than the C_4 species, *E. curvula* and the *Acacia* (**Tables 5.3 and 5.4**). There was no significant difference in C_3 grass mass between competition treatments with or without the *Acacia*. The C_4 grass, *E. curvula*, grown alone (5.8 ± 1.1g) accumulated more mass than when

grown with the seedling tree, (3.3 ± 1.4g). The *Acacia* accumulated significantly less mass when grown with the C_3 grass, than when grown with the C_4 grass, or alone (**Tables 5.1 and 5.3**). Interestingly, grass (C_3 and C_4) mass was greater when grown with rhizobially inoculated acacias than when grown with uninoculated trees (**Table 5.4**) across both CO_2 treatments.

Table 5.3 Average *Acacia* and grass fresh and dry masses (± std deviation) when grown in competition with one another. (* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$, n.s. not significant).

Combination of plants	Fresh mass (g)	*P*	Dry mass (g)	*P*
Tree mass				
With C_3 grass	2.2 ± 1.1		0.7 ± 0.3	
With C_4 grass	7.2 ± 2.8		1.9 ± 0.7	
Average across both grasses	4.7 ± 3.2		1.3 ± 0.9	
When grown alone	8.9 ± 2.7	***	2.3 ± 0.6	***
Average across all treatments in ambient CO_2, uninoculated	5.2 ± 1.9		1.4 ± 1.1	
Average across all treatments in ambient CO_2, inoculated	6.4 ± 1.7		1.7 ± 0.9	
Average across all treatments in elevated CO_2, uninoculated	6.2 ± 2.3		1.6 ± 0.7	
Average across all treatments in elevated CO_2, inoculated	6.6 ± 2.6	n.s.	1.8 ± 0.9	n.s.
C_3 grass mass				
With tree	10.3 ± 2.0		2.1 ± 0.5	
Without tree	10.3 ± 1.8	n.s.	2.1 ± 0.6	n.s.
C_4 grass mass				
With tree	3.3 ± 1.4		0.8 ± 0.6	
Without tree	5.8 ± 1.1	*	1.5 ± 0.4	n.s.
Average grass mass				
With tree	6.8 ± 3.9		1.5 ± 0.8	
Without tree	8.0 ± 2.7	n.s.	1.8 ± 0.5	n.s.

Across all treatments, when the tree seedling and grass masses were combined (total mass), the effect of CO_2, rhizobia and grass species were significant ($P = 0.001$). Rhizobial inoculation considered separately was insignificant. The interaction between CO_2 level and grass species ($P < 0.001$), and between rhizobial inoculation and grass species ($P < 0.05$) were also significant, but other interactions were not.

D. glomerata mass increased significantly (21%) when ambient growth was compared to growth under elevated CO_2. *E. curvula* mass increased by 6.5% while *Acacia* (grown alone) mass increased by 8% under elevated CO_2. When the C_3 grass and tree seedling were grown together, their combined mass increased from an average of 39.2g (fresh mass) in ambient CO_2 conditions to an average of 47.4g (21%) under elevated CO_2 mainly due to increased C_3 grass mass. Both the tree seedling and the grass increased in mass under elevated CO_2. Interestingly, when the C_4 grass and tree seedling were grown together their combined mass increased from 20.0g under ambient CO_2 to only 20.6g (3%) under elevated CO_2, mainly as a result of decreased *E. curvula* mass (also see **Table 5.4**) Analysed as a group, C_3 and C_4 grasses combined, there were no significant differences when trees were present or absent (**Table 5.3 and 5.4**). However significant differences were observed when the two species were analysed separately, across CO_2 level, in the presence or absence of the *Acacia* (**Table 5.6**).

Table 5.4 Average combined (four grass plant per pot) grass masses (± std deviation) of the C_3 and C_4 grass species, when grown under elevated (elev) or ambient (amb) CO_2 conditions, either with or without the (inoculated or uninoculated) *Acacia*. co-efficient of variation = 19.2% (fresh mass) and 25.6% (dry mass).

Treatment combination	Combined grass mass	
	Fresh	Dry
C_3 grass		
with tree, uninoculated, elev CO_2	44.7 ± 4.5	9.2 ± 0.4
with tree, uninoculated, amb CO_2	32.3 ± 6.1	6.5 ± 0.7
with tree, inoculated, elev CO_2	46.6 ± 4.9	10.6 ± 0.7
with tree, inoculated, amb CO_2	40.6 ± 5.8	6.6 ± 0.6
without tree, uninoculated, elev CO_2	46.1 ± 4.2	10.3 ± 0.9
without tree, uninoculated, amb CO_2	36.2 ± 6.4	6.3 ± 0.8
C_4 grass		
with tree, uninoculated, elev CO_2	10.0 ± 1.9	2.4 ± 0.6
with tree, uninoculated, amb CO_2	12.5 ± 2.4	3.2 ± 0.8
with tree, inoculated, elev CO_2	15.0 ± 2.9	4.2 ± 1.1
with tree, inoculated, amb CO_2	15.1 ± 2.9	3.7 ± 0.9
without tree, uninoculated, elev CO_2	21.0 ± 2.6	5.4 ± 0.9
without tree, uninoculated, amb CO_2	25.4 ± 4.6	6.8 ± 1.6

When each factor was considered in combination with another, an interesting pattern emerged. Elevated CO_2 increased *Acacia* plant mass in the absence of rhizobia (by 16%), and rhizobial inoculation increased *Acacia* mass in ambient CO_2 by 18%. Carbon dioxide barely increased (3%) *Acacia* mass in the presence of rhizobia while rhizobial inoculation

marginally increases *Acacia* mass (5%) in the presence of elevated CO_2. The greatest overall *Acacia* mass was achieved with elevated CO_2 and rhizobial inoculation. The presence of a grass greatly decreased tree seedling mass. This decrease was greater under elevated CO_2, as the accumulation in mass under elevated CO_2 was greater. The greatest *Acacia* mass for this combination was acquired under elevated CO_2 with no grass, and secondly with the C_4 grass, *E. curvula*, (**Tables 5.1, 5.3 and 5.5**) while plants grown with the C_3 grass (*D. glomerata*) had the smallest mass. Elevated CO_2 had a minor non-significant effect on individual tree seedling mass when grown with a grass plant (averaged over the two species), increasing the tree seedling mass by an average of 9%. Interestingly, when grown with the C_3 grass, ambient CO_2 concentrations and rhizobial inoculation produced the greatest mass, while elevated CO_2 and no inoculation resulted in the lowest *Acacia* mass, although these interactions were not statistically significant. When grown with the C_4 grass, the presence of both factors, (elevated CO_2 and rhizobial inoculation) resulted in the greatest *Acacia* mass, and the absence of both, the lowest *Acacia* mass (**Table 5.5**).

Rhizobial inoculation had a small but significant effect on tree seedling mass when grown with grass plants increasing the tree seedling mass compared to uninoculated plants by 12% (**Tables 5.1 and 5.5**). When the combined effects of rhizobial inoculation and grass presence/absence are analysed, rhizobia increased mass, and the presence of a grass decreased *Acacia* mass. Rhizobial inoculation increased *Acacia* mass more when grown with the C_4 grass than when grown with the C_3 grass.

When each factor (CO_2 and rhizobia) was compared individually to the plant mixture treatment (tree and grass mix), it is seen that elevated CO_2 increased the growth (mass) of the acacias the most when grown with the C_4 grass (mass increased by 32% (fresh) /36% (dry)). Elevated CO_2 improved *Acacia* mass by only 8%(fresh)/4%(dry) when the trees were grown alone. Perhaps most significantly, elevated CO_2 decreased *Acacia* mass by 37% (33% dry) when grown with the C_3 grass (**Table 5.5**). Rhizobial inoculation had a beneficial effect on *Acacia* growth in all the treatments, although this effect was only minor when the tree seedling was grown with the C_3 grass. Rhizobial inoculation increased *Acacia* dry mass by 12% /19% (fresh/dry) when grown with the C_3 grass, by 28%/24% when grown with the C_4 grass and by 4%/2% when grown alone ($P \leq 0.0001$).

Table 5.5. The interactive effect of a competing grass species on total *A. sieberana* fresh and dry masses (± std deviation) with either CO_2 level (elev/amb) or rhizobial inoculation, or the interaction of CO_2 level and rhizobial inoculation. (* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$, n.s. not significant).

Factor	Fresh	*P*	Dry	*P*	Interaction *P*
Tree mass with C_3 grass					
CO_2 level					
Elevated	1.7 ± 1.0	***	0.5 ± 0.3	*	
Ambient	2.8 ± 0.9		0.8 ± 0.2		
					n.s.
Rhizobial inoculation					
Inoculated	2.37 ± 1.1	n.s.	0.6 ± 0.3	n.s.	
Uninoculated	2.11 ± 0.9		0.7 ± 0.3		
Tree mass with C_4 grass					
CO_2 level					
Elevated	8.2 ± 2.6	*	2.2 ± 0.7	*	
Ambient	6.2 ± 2.6		1.6 ± 0.6		
					n.s.
Rhizobial inoculation					
Inoculated	8.1 ± 3.2	*	2.2 ± 0.9	*	
Uninoculated	6.3 ± 1.8		1.7 ± 0.4		

Table 5.6 The effect of CO_2 concentration (elev/amb) the presence or absence of an *Acacia*, presence/ absence of rhizobial inoculation, and grass species (C_3 or C_4), on averaged grass mass. An analysis of variance was carried out for each grass species (* $P < 0.05$, ** $P < 0.01$, n.s. not significant, dry mass coefficient of variation 25.6%, fresh mass, cv 19.2%).

Treatment	Factor	Dry mass	Fresh mass
Presence / absence of a tree		**	**
No tree present			
	CO_2 level	*	n.s. (0.119)
	Grass species	**	**
	CO_2 x grass species	**	**
Tree present			
	CO_2 level	**	**
	Inoculation	*	**
	Grass species	**	**
Interactions			
	CO_2 x inoculation	n.s. (0.07)	n.s. (0.424)
	CO_2 x grass species	**	**
	Grass species x inoculation	n.s. (0.583)	n.s. (0.619)
	Interaction of all factors	n.s (0.969)	n.s. (0.082)

5.4 Discussion

Since tree seedling mass was greater when the seedlings were grown alone, and this mass decreased when they were grown with the grasses, it can be assumed that a competitive effect was operating between the species combined at any one time. Because rhizobial inoculation increased tree seedling mass and elevated CO_2 increased plant mass in both the trees and grasses it might be assumed that competition for a finite amount of resources also increased with rhizobial inoculation and elevated CO_2 (**Table 5.1**).

Grasslands are most often dominated by species having the C_4 photosynthetic pathway, whereas woody encroaching species are generally C_3 (JOHNSON *et al.* 1993; WAND *et al.* 1996). Owing to their specialised photosynthetic mechanisms C_4 species generally exhibit a smaller photosynthetic growth stimulation in elevated CO_2 than C_3 species (RAWSON 1992; ARP *et al.* 1993; WAND *et al.* 1996). The results of this experiment seem to support the validity of these statements in that C_3 grass species (*D. glomerata*) fared 77.6% better than the C_4 species (*E. curvula*) in terms of mass acquisition (**Tables 5.2, 5.3, 5.4 and 5.6**). The difference between grass species when grown with or without the *Acacia*, at elevated and ambient concentrations of CO_2 was significant, mainly due to responses of the C_4 grass, since the C_3 grass was little affected by the presence of the *Acacia* (**Table 5.4**) when determined by Analysis of Variance.

The presence or absence of the competing species (grass or tree) had a substantial effect on the final masses of both the grasses and the tree seedling, although this effect was most pronounced in the *Acacia* and *E. curvula*. The C_3 grass accumulated significantly more mass than the C_3 *Acacia*, although this mass was decreased in the presence of the tree seedling, and increased by elevated CO_2 and inoculation. The C_4 grass also increased in mass when grown with an inoculated tree seedling, as compared to an uninoculated tree seedling, but gained the most mass when grown alone, uninoculated, in ambient CO_2 (**Table 5.4**).

The tree seedling was most affected by the presence of the C_3 grass, decreasing in average mass from 8.9g to 2.2g. This decrease, when grown with the C_4 grass was only 1.7g (decreased from 8.9g to 7.2g), which was above average (4.7g) for the treatment. The

Acacia mass decreased from an average mass of 6.4g in elevated CO_2 to 5.8g in ambient CO_2 when averaged over both grass species and although this was not significant, the interaction between the grass species and CO_2 on tree mass was significant (**Table 5.1**). None of the other interactions were significant. Thus the prediction that grasslands may become more woody may be some what premature, unless such a woody plant can establish and maintain itself in an area of disturbance, where no competing grasses exist. From personal observations this would appear to be unlikely, as grasses and weeds are often most dominant in such positions. Nevertheless, plants with K life strategies, such as this *Acacia*, may dominate after the lengthy process of succession. Once this happens it becomes very difficult for the grasses to re-establish.

In natural communities, CO_2 enrichment could alter species composition by changing the interaction of coexisting species (BAZZAZ and MCCONNAUGHAY 1992; JOHNSON *et al.* 1993; STEWART and POTVIN 1996). Although statistically different, *Acacia* mass when grown with the C_4 grass was quite similar to the mass of the trees grown alone, but very different to the mass of the trees when grown with the C_3 grass. Thus it might be extrapolated that these trees will fare better when growing naturally in competition with C_4 grasses than when growing near C_3 grasses. Because most grass species (especially in southern Africa) are C_4, there may be increased pressure on our grasslands and savannas, to tend towards a more woody (C_3) canopy (ARCHER *et al.* 1988; ARCHER 1990). This is undesirable in that it may result in decreased grazing capacity and soil stabilisation, which in turn corresponds to decreased erosion prevention. It should be noted that this experiment was conducted under relatively low light conditions, favourable to the C_3 grass. This may have contributed to the superiority of the C_3 grass over the other two species. Under field conditions, increases in woody C_3 plants may increase available shade, and result in further increases in C_3 plants, perhaps increasing numbers of smaller plants like C_3 grasses and herbs.

Nitrogen fixing species exhibit a greater growth stimulation in elevated CO_2 than non-N_2 fixing species, due to greater availability of carbohydrates to sustain the high-energy demands of N_2 fixation (NORBY 1987; WAND *et al.* 1996). This may provide encroaching woody species, many of which are nitrogen-fixing legumes with an added advantage (WAND *et al.* 1996). Therefore, the presence of rhizobia in the soil will further aid the growth and development of nitrogen fixing species such as acacias in an elevated CO_2 environment. This will improve the competitive ability of these plants as demonstrated in

this experiment and further decrease the likelihood of C_4 grass dominance. Also, the relatively greater effect of elevated CO_2 on nitrogen fixing species, when nodulated, (**Table 5.3**) demonstrates the expression of competitive life history traits, and is consistent with the pattern obtained by MIDGLEY *et al.* (1995), with species from higher nutrient status soils. Elevated CO_2 increased *Acacia* mass more when inoculated, but the effect of inoculation on plant mass grown under ambient CO_2 conditions was almost as great. Neither was significant, but this may have been because the tree seedling mass was averaged across all competition combinations. Also, the failure of nodulated plants to perform significantly better may have been because light (rather than nitrogen) limited growth.

It seems that the photosynthetic apparatus which has evolved in C_4 plants was developed as a special adaptation for photosynthesis at low CO_2 concentrations, when CO_2 was only a trace gas in the atmosphere (KRAMER and BOYER 1995). The general hypothesis is that increasing CO_2 levels will shift the competitive balance in favour of C_3 species. However, interaction between elevated CO_2 and other environmental factors may alter the CO_2 response (BAZZAZ and MCCONNAUGHAY 1992; JOHNSON *et al.* 1993; WAND *et al.* 1996). OWENSBY *et al.* (1993, cited in WAND *et al.* 1996) found substantially greater increases in the growth of C_4 species than in C_3 grass species during a year with water stress, resulting in improved competitive ability of the C_4 species (WAND *et al.* 1996). Low light may also have affected the results obtained in this study (NIJS and IMPENS 1996) C_3 plants usually benefit to a greater degree, and demonstrate enhanced growth under low light. However, WONG and OSMOND (1991, cited in BAZZAZ and MCCONNAUGHAY 1992) found the opposite result. Metabolic changes, particularly in photosynthesis, which occurred during the course of evolution and improved plants' ability to withstand limited water supplies were especially well developed in C_4 and CAM plants (ROGERS and DAHLMAN 1993; KRAMER and BOYER 1995). Nutrient limitations may also mitigate the potential CO_2 response over the medium to long term, thus the importance of the nitrogen fixing mechanism (BAZZAZ and MCCONNAUGHAY 1992).

Furthermore, it is worth mentioning that individual plants do not actually compete for CO_2. It is unlikely that the CO_2 supply for one type of plant might be diminished sufficiently by the CO_2 use and uptake of another plant, to make a difference in the usual sense of competition. The composition of the atmosphere is highly 'conservative' as a consequence of its large volume and vigorous mixing dynamics (JOHNSON *et al.* 1993). The effects of

changing CO_2 concentrations on species composition will not be mediated through competition for CO_2 *per se* but will depend primarily on how the rate of carbon supply influences individual growth rates and alters acquisition and utilisation of other required resources (BAZZAZ and MCCONNAUGHAY 1992).

In this experiment, differences in the growth rates and responses to experimental conditions of light and nutrients (as well as other resources) in the C_3 and C_4 grasses and the C_3 tree resulted in differences in height and biomass acquisition. This can be viewed as a change in the ability to compete for these resources. An increased carbon supply can be expected to affect species differentially through changes in: 1) the rates of growth; 2) the proportions and absolute amounts of specific growth resources required for completing lifecycles and insuring ecological success; 3) the rates at which scarce essential growth resources can be acquired; and 4) the actual habitat requirements (JOHNSON *et al.* 1993). As a result, when grown under natural conditions, the C_3 grass in this experiment would be expected to fare better than the other two species, especially initially, before the C_3 tree seedling became established, and acquired the help of root symbionts like *Rhizobium* sp. After this the additional help of these nitrogen-fixing bacteria would supply an advantageously higher amount of nitrogen to the legume. The C_3 grass might also be expected to fare better than the C_4 grass in the shade of the *Acacia*, a specific habitat adjustment. However, the C_4 grass may grow better than the other two species in dry conditions, because it makes better use of the water resource.

Thus, all three plant types show some potential for survival in a future hotter, elevated CO_2 environment. However, water stress has repeatedly been observed to be ameliorated by increased concentrations of CO_2, this may be due to partial closure of stomata (ROGERS and DAHLMAN 1993). This protection from water stress may extend to both C_4 and C_3 plants, further helping to relieve negative impacts of the future drier climates, but complicating prediction further.

5.5 Conclusions

Comparing the responses to nitrogen and CO_2 of these C_3 and C_4 plants demonstrates that responses cannot be generalised. Within these species there are different temperature optima, varying degrees of scope for changing organ size and number and partitioning of

carbon within and between organs. Furthermore, there are differing responses of growth to competition and rhizobial inoculation, without even adding CO_2 as a further variable. WAND *et al.* (1996) concluded that they could not base their predictions of C_3 and C_4 plant response to climate change purely on photosynthetic mechanisms. This experiment has highlighted the myriad of possible combinations of interactions within and between plants and their environments. The importance of exact experimental procedure is emphasised by the many plausible explanations of the results. Future experiments must be designed with as few variables and in as natural an environment as possible.

The results obtained in this study suggest that C_3 plants, especially the grass *Dactylus glomerata* will fare markedly better than their C_4 competitors. A grass like *Eragrostis curvula*, which is usually extremely competitive under all conditions, was out-competed by the C_3 grass. C_3 grasses are not common in southern Africa, and may therefore not be able to fill the niche opened by the demise of the C_4 grasses. However, this experiment was conducted under relatively low light conditions, favourable to the C_3 grass. This may have contributed to the superiority of *D. glomerata* over the other two species. If however, C_3 trees do come to dominate future landscapes, there will be more shade, and thus potentially more opportunity for these grasses. This may facilitate the establishment of presently uncommon plants, even in the absence of growth enhancements.

Differences in absolute rates of photosynthesis do not by themselves dictate vegetation composition and structure, as evident in present day vegetation, where many species co-exist in the same community though they have very different absolute rates of carbon uptake. While these results show that C_3 plants receive more benefit from increased CO_2 than C_4 plants, it is important to note that at the individual species level pronounced differences in response may occur within each functional type. Some C_4 species may even benefit from rising CO_2.

Since plants seldom compete for CO_2 directly, the overall effect of elevated CO_2 on plant growth and competition will be minimal in comparison to other plant resources. CO_2 availability is likely to alter plant interactions indirectly through its effects on growth and architecture, and thus the acquisition of other more limiting plant resources.

CHAPTER 6

The effect on growth and survival of transplant seedlings of *Acacia nilotica* and *Acacia sieberana* at three different elevations at Cathedral Peak

6.1 Introduction

In a future elevated CO_2 world, several factors will be crucial in determining the rate at which species distributions change. A species can extend its range only if: a) humans move it or b) by natural processes seeds are dispersed to, and seedlings establish in, areas beyond their current distribution (ANTONIO and VITOUSEK 1992; KING and NEILSON 1992; LEISHMAN *et al.* 1992). Migration rates of about 30-100km per decade will be required for plants to remain in their present climatic envelopes (LEISHMAN *et al.* 1992). These authors and WELLS (1983) also conclude that long-distance dispersal events will be very important in determining migration rates and species dispersal possibilities.

The kinetics of Rubisco indicate that CO_2 enrichment will enhance photosynthesis relatively more as temperatures increase. Thus C_3 plant productivity may be further enhanced under elevated CO_2 at warmer temperatures (HUNT *et al.* 1996). This will further contribute to the invasion of grasslands (predominantly C_4) by woody C_3 plants. The present study considers the effect which rhizobial inoculation might have on *Acacia* growth and hence distribution in an elevated CO_2 environment. While the predicted *specific* increase in temperature has not been fully quantified, the upward trend does exist, and increasing CO_2 levels will translate to increases in temperature. Therefore migration with altitude (in order to stay within the present temperature envelope) is investigated as a likelihood. The aim of this study was to determine the effects of altitude and altitude associated temperature changes on *Acacia* growth. Since rising concentrations of carbon dioxide mean rising temperatures, the effect of likely changes in ambient temperature on the performance of acacias was to be tested, and since temperature decreases with altitude, transplant experiments were carried out. While the altitudinal and associated

temperature effects were studied in the field and are covered in this Chapter, the rhizobial and CO_2 effects were dealt with in greenhouse experiments (in previous Chapters). Although it is not ideal to separate the study factors to this degree, it was the chosen and most practical course of action considering the budget and time constraints. This should therefore be considered as one of the limitations of the present study.

Acacia sieberana is one of the most common *Acacia* species in southern Africa, and is a large deciduous tree widely branching and sometimes reaching 17m in height; although it can only be about 7m in height with a comparatively immense spread. It occurs in woodland and wooded grassland / savanna, often along rivers or on flood plains. It is not often found at high altitudes, where the temperature can decrease to levels too low for this species, and for *A. nilotica*. *A. nilotica* can reach 10m in height, but is usually smaller, occurring in a variety of woodland, savanna and scrub, frequently forming thickets (COATES PALGRAVE 1995).

6.2 Materials and Methods

To determine the possible reasons for current *Acacia* dispersal patterns, as well as probable future patterns a number of *Acacia* seedlings were transplanted within and above their current elevational distribution limits. Twenty plants of each of *A. sieberana* and *A. nilotica* were planted at three sites (120 plants in total) at Cathedral Peak in the KwaZulu-Natal Drakensberg. Seeds of *A. nilotica* and *A. sieberana* were obtained from the same sources as those used in the previous experiments - from plants growing in and around the suburbs of Bisley and Hayfields in Pietermaritzburg, KwaZulu-Natal. The first was at 1410 metres above sea level (masl), their current distributional level, the second at 1850 masl, at the upper limit of their current distribution. The third site was at the Brotherton plots, 1915 masl in what appeared to be extreme conditions for these species, characterised by open grassland and strong winds (**Figures 6.1 and 6.2**). Saplings were not planted at elevations below 1410 masl, as they commonly occur at those elevations. The sites were allocated to this experiment by the (then) Natal Parks Board (KwaZulu-Natal Wildlife), based on climate, elevation, rainfall and aspect requirements. The sites were chosen in close proximity to one another in order to reduce climate and rainfall variability. An attempt was made to obtain sites of similar aspect, but this was not entirely possible due to the assignment of the sites.

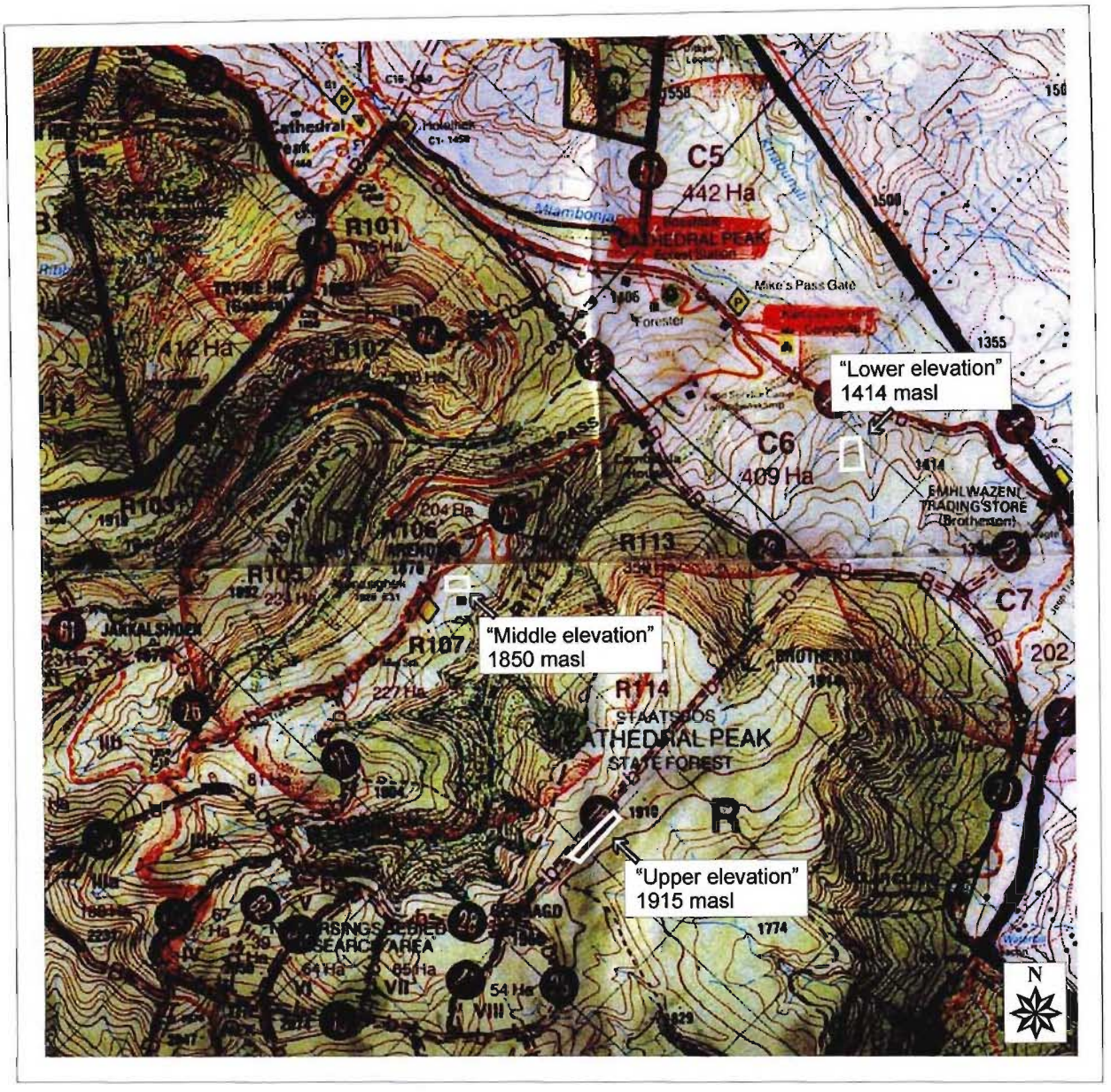

Figure 6.1. Location map - Cathedral Peak Nature Reserve.The positions at which the three different elevational treatments were located, and the terrain encountered.

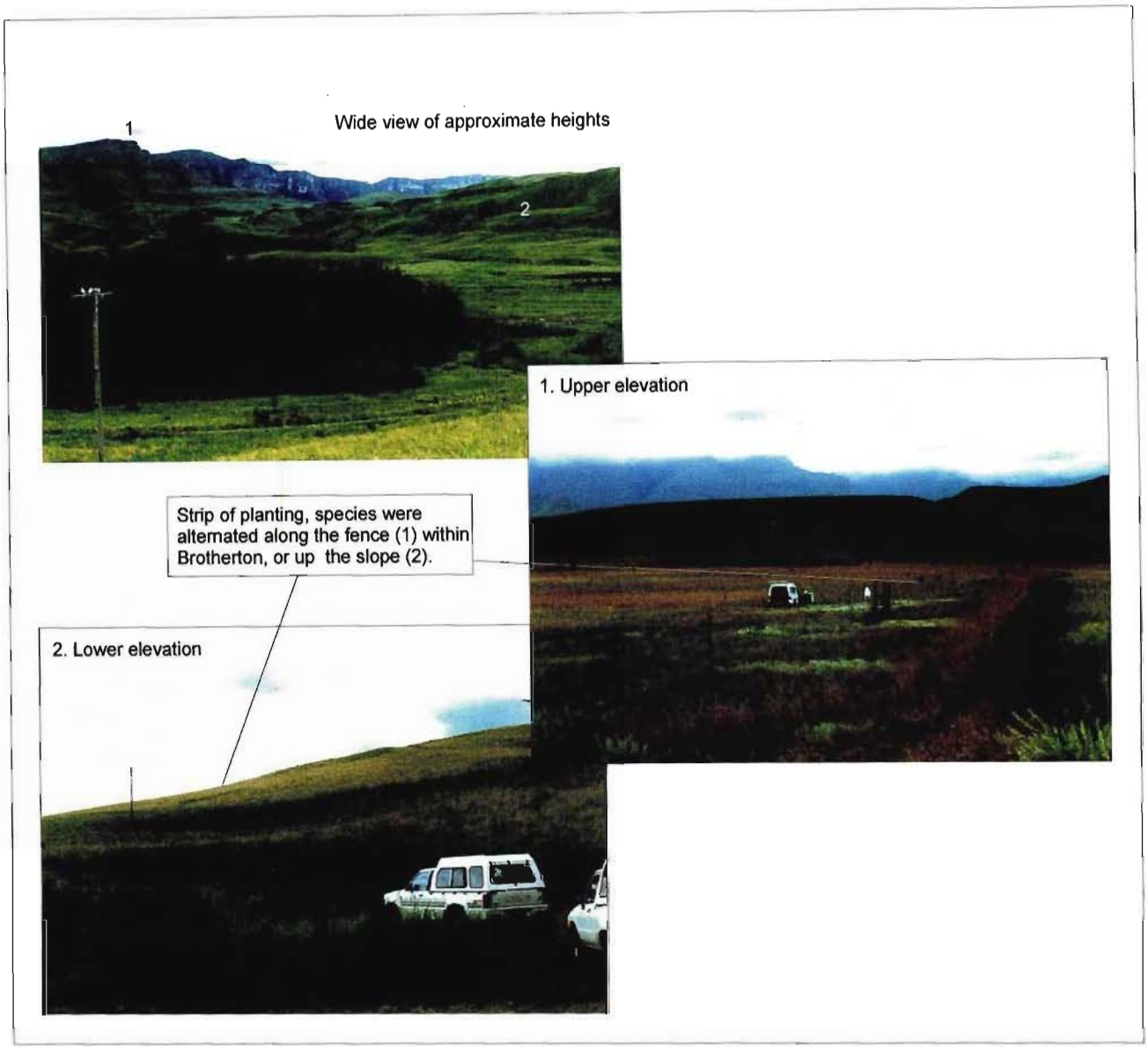

Figure 6.2. Sites of upper and lower elevation planting. Inserts (1 and 2) show actual sites, and wide view the approximate heights (but not locations) of these elevations when compared to the view across the valley.

Figure 6.3. A newly planted sapling. Plants were not watered. Only a small area of vegetation was disturbed to plant each tree.

Figure 6.4. *Acacia sieberana* after winter. Plants at the upper elevation (A) were more severely affected by the cold, wind and frost, but some still recovered. Plants at the lower elevation (B) flourished throughout the experimental period.

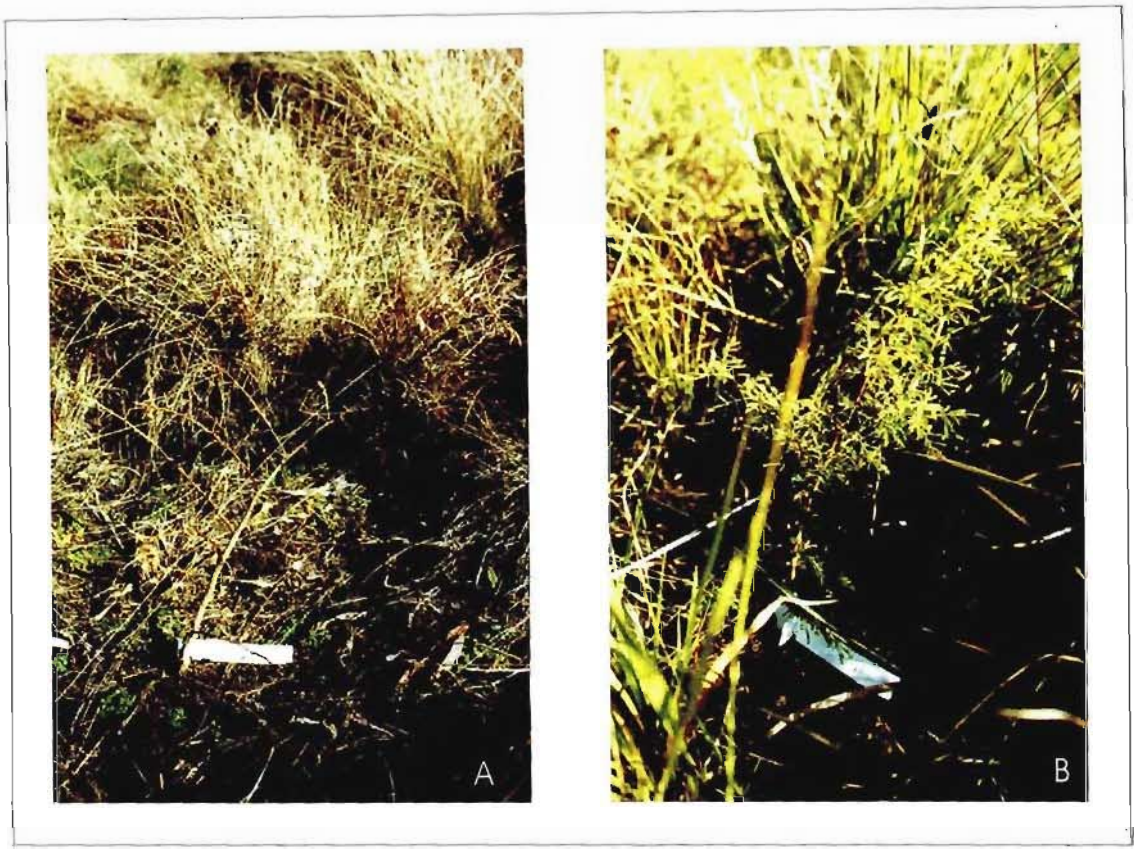

Figure 6.5. *Acacia nilotica* after winter. *Acacia nilotica* did not appear to be as hardy or grow as fast as *Acacia sieberana*, but some plants grown at the upper elevation (A) recovered after winter, while those grown at the lower elevation (B) had regained their leaves soon after winter. A middle elevation specimen is not shown here as the response was similar to the lower elevation, only with less growth.

The plants (height) were measured upon planting, to establish a baseline (early summer, **Figure 6.3**), and were measured again in autumn. Upon termination of the experiment (after 14 months, during mid summer) the comparative health (visually judged) of each plant was noted on a scale of 0 - 5 (with 5 being the best possible condition and 0 the worst). A value of 5 was assigned to plants considered to be in excellent condition (no leaf loss or discolouration), and 4 to plants in which some (1 - 15 %) of the leaves had been lost or discoloured. A value of 3 was assigned to plants which had lost/discoloured 16 - 30 % of their leaves, 2 to plants which had lost/discoloured 31 - 50 % of their leaves and/or some branches; and 1, to plants which had lost/discoloured 51 - 80 % of their leaves and/or branches. The value of 0 was always reserved for plants with no leaves (apparently dead) although leaf loss percentages from 81 - 100 % were also assigned this value.

The plant heights were not recorded upon harvesting, although they were noted. Most of them had decreased substantially in size due to die back, and had also split into many separate stems. Plants at the third site, at Brotherton, had died back considerably during winter. Many plants of *A. nilotica* did not recover. Some of the *A. sieberana* plants re-sprouted in summer. A few (*A. sieberana*, 2; *A. nilotica*: 1) of the plants at this upper site were also completely removed or damaged by baboons, and these were excluded from the results. *A. nilotica* plants grown at the middle site were mostly dead, while *A. sieberana* plants were mostly alive. At the lower site all the plants flourished. Only above-ground biomass was harvested, and of this, only dry mass was measured, due to the remoteness of the sites from weighing apparatus. It was not possible to efficiently or effectively harvest the entire below ground root mass, as these plants have substantial a tap root and a fibrous root system. Consequently, the roots were not harvested due to the inaccurate results which would have been obtained. Plants were dried at 75-80°C for 48 hours, and then weighed. Survivorship, probable causes of mortality, and comparative growth rates between the groups were noted.

6.3 Results

6.3.1 Plant height

Acacia sieberana grown at different elevations showed no significant differences between the treatments (**Table 6.1**). The height of *A. nilotica* had increased significantly more at the

lower elevation compared to the upper elevations ($P = 0.05$). The lower elevation increase in height was significantly different from the other two treatments ($P = 0.005$). The large deviations in mean values as seen in Table 6.1 are unfortunate, and may have been caused by small differences in microclimate (immediate proximity to and availability of nutrients), as all measuring techniques were considered highly uniform. Some trees just did not grow while others grew exponentially. The starting standard deviations (for height) were on average 78.1mm, 74.5mm and 76.8mm for *A. sieberana* lower, middle and upper elevations, and 64.0mm, 74.3mm, and 49.3mm for *A. nilotica* lower, middle and upper elevations respectfully.

Table 6.1. The effect on height, mass and general condition of *Acacia nilotica* and *Acacia sieberana* grown at three different elevations for one year. The condition ratings were visually assigned to the plants, and were from 0 to 5 (with 5 being the best possible condition and 0 the worst) (* $P < 0.005$, ** $P < 0.001$, n.s. not significant masl: metres above sea level).

Elevation	Increase in plant height (mm)		Mass (g)		Condition	
A. sieberana						
1414 masl: '0'	126.2 ± 117.8		17.3 ± 9.6		3.7 ± 0.7	
1850 masl: '1'	68.3 ± 81.3	n.s.	3.6 ± 2.0	**	3.9 ± 0.8	**
1910 masl: '2'	85.3 ± 84.2		2.0 ± 1.5		0.8 ± 0.9	
A. nilotica						
1414 masl: '0'	71.2 ± 48.2		10.2 ± 3.5		4.3 ± 0.6	
1850 masl: '1'	32.7 ± 41.7	*	4.7 ± 2.4	**	3.4 ± 0.6	**
1910 masl: '2'	33.1 ± 28.1		3.1 ± 1.0		0.3 ± 0.4	

6.3.2 Plant mass

At the end of the experimental period the mass of the plants at the lowest altitude (1414 metres above sea level) was greatest in both species ($P < 0.001$). The two higher elevations, 1850 masl (middle), and 1910 masl (upper) had very similar masses but very different condition ratings (**Table 6.1**). Baboons may have interfered with the plants at the higher elevation, damaging some of them. Some of the plants were removed and had lost their leaves, but the labels were still attached and the identity of plant could therefore be determined. Therefore while the masses of the plants at the upper and middle elevations were similar, due to the presence of the main stems and some branches, the loss of leaves reduced the condition rating considerably. This may have been due to animal activity, or, due to extremes in temperature and wind conditions. Plant mass increased by an average

of 765% and 223% at the upper and lower elevations in *A. sieberana* and *A. nilotica* respectively.

6.3.3 Plant condition comparisons

Plants were compared visually and assigned a value from 0 to 5 (**Table 6.1. and Figures 6.4 and 6.5**). *A. nilotica* appeared to be in a better overall condition compared to *A. sieberana*. Plants of both species grown at the lower and middle altitudes were in a much better condition than those grown at the upper elevation. There was a significant difference between all the *A. nilotica* treatments, with the lower elevation producing plants in the best condition and the upper elevation the worst condition ($P < 0.0001$). *A. sieberana* plants grown at the middle and lower elevations were in a similar condition but were significantly different from those grown at the upper elevation ($P < 0.0001$).

It should be acknowledged that the acacias were planted among plants (mostly grasses) adapted to the environmental conditions at the study site. Thus, transplanting an *Acacia* beyond its current elevational range it is forced to compete with plants adapted to these conditions, and competition will increase.

6.4 Discussion

Different altitude regimes and the associated temperature and other altitudinal effects (solar radiation, wind speed, wind chill, and even animal abundance and species type, which differ with the increase in altitude) had a significant effect on all three characteristics measured in this experiment. Small differences in aspect could have affected the results between the sites, but this is unlikely since all the sites were relatively equally exposed, and as 'flat' as was possible to obtain. Plant height, condition and mass all decreased with the increase in altitude. This was a simulated attempt at predicting how plants might respond to changes in global temperature regimes. In mid-continental Australia a 1°C change in temperature corresponds to a latitudinal distance of 100 - 125km. If similar temperature distance relationships are assumed in the future, a 3°C rise will lead to a 300 - 372km displacement of isotherms towards the poles during this century (IPCC 1990; cited in LEISHMAN *et al.* 1992). If it is presumed that mean temperature decreased with altitude in the Cathedral Peak area in the time over which this experiment was conducted, a rough

estimate of the effects of the predicted climate change regime on *Acacia* growth is obtained.

Other important factors (rainfall, dew, frost, wind etc.) may also have changed within this small envelope in time and space, but these are considered as part of the overall picture in terms of altitudinal/latitudinal migration. Therefore, while these other factors are likely to influence *Acacia* distribution to a greater degree than a 1 or 2 degree centigrade change in temperature, the effect of rhizobial inoculation and CO_2 concentration cannot be disregarded. In the case of rhizobial inoculation especially, it should be considered at the forefront of plant distribution prediction. Plant growth was most successful at the lower elevation. It is likely therefore, considering these results, that if temperature was the main determining factor, with all else being equal, as temperatures increase, the distribution range of acacias will increase with altitude and latitude.

The condition ratings between the middle and lower elevations were very similar, therefore indicating the ability to grow and produce healthy plants, albeit smaller plants at the higher altitude. Also, the very fact that some of the plants at the upper altitude were able to recover after a harsh winter and produce healthy summer growth is an indication of the hardiness of these plants (**Figures 6.4 and 6.5**). The main obstacle to future range expansion might however be distribution of the seed, and overcoming the problem of seed dormancy. The variation between species migration rates implies that some rates will be limited by dispersal rather than by the rate of climate change (LEISHMAN *et al.* 1992).

The effects of human-caused biological invasions are long-term, whereas changes in climate, the atmosphere and landuse may be reversible in hundreds to thousands of years. The breakdown of biogeographic barriers has resulted in self maintaining and evolving populations in regions they might otherwise not have reached. This change is considered irreversible (ANTONIO and VITOUSEK 1992; SCHLESINGER 1993). The distribution of root bacteria such as the rhizobia that infect *Acacia* roots should then be considered very important in determining the success of any invasion by an *Acacia* species. As demonstrated in Chapter 4, the presence of active root nodule bacteria was the most influential factor in deciding the success of *Acacia* plants. This may be the most important consideration in future predictions regarding *Acacia* distribution in an elevated CO_2

environment. Any further experiments should also determine the presence of root nodule bacteria (or their effects) in the soil.

Further factors for consideration are the effects of competition and the type of CO_2 pathway (C_3, C_4 or CAM, see Chapter 5) on plant growth and distribution. Grasslands are most often dominated by species having the C_4 photosynthetic pathway, whereas woody encroaching species are generally C_3 (JOHNSON *et al.* 1993; WAND *et al.* 1996). Owing to their specialised photosynthetic mechanisms C_4 species generally exhibit a smaller photosynthetic growth stimulation in elevated CO_2 than C_3 species. This will again alter the competitive balance between the majority of grass and tree species (ARP *et al.* 1993; JOHNSON *et al.* 1993; WAND *et al.* 1996; MIDGLEY "50/50 SABC TV3" 1999). The general hypothesis is that increasing CO_2 levels will shift the competitive balance in favour of C_3 species. The results obtained in this study (Chapter 5) also seem to indicate that C_3 species may fare better than C_4 species. Interaction between elevated CO_2 and other environmental factors as previously mentioned may however alter the CO_2 response (JOHNSON *et al.* 1993; WAND *et al.* 1996).

Nitrogen-fixing species exhibit a greater stimulation in elevated CO_2 than non-N_2 fixing species, due to greater availability of carbohydrates to sustain the high-energy demands of N_2 fixation (NORBY 1987; WAND *et al.* 1996). This may further aid encroaching woody (ARCHER *et al.* 1988; ARCHER 1990) species (C_3), many of which are nitrogen-fixing legumes (WAND *et al.* 1996). Although species richness might not differ, the composition of communities may change. CO_2 enrichment may alter the composition of communities by changing the interactions between co-existing species (STEWART and POTVIN 1996)

6.5 Conclusions

This experiment was not ideally designed to address the questions it set out to answer. Too many variables were apparent in the experimental conditions up to deduce any conclusive answers to the problem presented. It can be said however, that some factor which was probably temperature or wind chill related, affected the growth of acacias negatively at the upper elevations. This factor may be ameliorated with a future increase in CO_2 concentration that will also be accompanied by increases in temperature. The results of this experiment indicate that *if* an elevated atmospheric CO_2 response is

associated with a temperature and/or altitude response, this *will have* a statistically significant positive effect on growth.

Furthermore, based on the results of this study (in earlier chapters) if Rhizobia are present in the soil the invasion of acacias into new areas will be facilitated further. If the performance of plants that photosynthesize via the C_3 pathway is increased by CO_2 enrichment as predicted by this and other studies, woody plants may encroach into grasslands and savannas. These areas may become densely wooded, and this may lead to increased erosional problems as a result of reduced basal cover. Under this scenario there will also be a reduction in the quantity and quality of available grazing, which has many implications for both animal and plant life.

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